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COMPARATIVE EXPERIMENTS WITH THE PROLONGED ADMINISTRATION OF α AND β OESTRADIOL

Failure of β-oestradiol to act as an antagonistic analogue of α-oestradiol

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INTRODUCTION

IN WORK with antifibromatogenic 3-keto-steroids by whose administration oestrogen-induced abdominal fibroids can be prevented in the guinea pig, two relevant conclusions as to their mode of action were reached: *first*, antifibromatogenic steroids do not act by the intermediance of the gonadotrophic system of the hypophysis since they act both in intact and *castrated* females; and *secondly*, antifibromatogenic activity of progestational, cortical, or androgenic steroids is not a sequel of their known, or classical, physiological faculties, but a faculty *per se*, independent of the former. In an earlier phase of our work we postulated that antifibromatogenic action was intimately related to progestational faculty (Lipschutz, [5]) since progesterone is the most potent antifibromatogenic steroid and since potency decreases with desoxycorticosterone, dehydrocorticosterone, testosterone, dihydrotestosterone. However, it has been shown later on that androgenic steroids whose progestational activity is increased by substitutions at C_{17} do not acquire by this greater antifibromatogenic activity (Iglesias and Lipschutz [3]; Lipschutz, Iglesias, Bruzzone, *et al.* [8]) Neither is the antifibromatogenic action related to androgenic or corticoid activity (Iglesias and Bruzzone [2]). This is why the tentative assumption was made that antifibromatogenic steroids interfere in some uniform way in the growth process of cells stimulated by oestrogen (Lipschutz,[6]; Lipschutz *et al.*[8]).

The above assumption of an interplay of the antifibromatogenic steroid with the oestrogen in the tissues or cells affected by the latter was but an extension of the old concept of the peripheral antagonism of sex hormones (see Lipschutz [7], p. 125-127, 157). However, so far, there was no direct experimental statement to substantiate it. In experiments of

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Bruzzone and Toro ([17]) it was tried to localize antifibromatogenic action in a given fibromatogenic area by implanting a tablet of progesterone into the spleen and thus making it to circulate only in the latter, whereas a tablet of oestrogen was implanted beneath the skin; yet no such localized antifibromatogenic action could be demonstrated in these experiments (for the device of Bruzzone and results, see also [7], p. 157-158). But neither was there, so far as we know, any experimental statement contrary to the above assumption of antifibromatogenic steroids acting directly on the tissues subject to the tumorigenic action of the oestrogen. Progesterone, for instance, has not been found to increase, but to decrease the hepatic inactivation of oestrogen (Segaloff, [14]); this would tend to increase rather than to decrease the tumorigenic effect of the oestrogen.

The concept of a peripheral interplay between the antifibromatogenic steroid and the oestrogen being as yet the only viable explanation of antifibromatogenic activity of steroids, another auxiliary assumption also was made: that antifibromatogenic steroids act as inhibitory analogues of oestrogens. It was consequently thought that β-oestradiol as an isomer of α-oestradiol of very weak estrogenic action should be tried as to antifibromatogenic activity *. Indeed, β-oestradiol is not void of oestrogenic activity though the latter is a weak one (Selye [16], cpd. N° 36; also p. 12, 13 and 21); β-oestradiol is also inactivated in the liver (Segaloff, [15]). All this was, from the onset, to render the situation rather contradictory. The other known isomer, iso-oestradiol, is of so considerable an oestrogenic actitvity (summary Selye, [16]) that it did not enter into consideration in our work at all.

Experiments with the administration of β-oestradiol

The first step to be made was a comparative study of the actions of β-oestradiol and α-oestradiol when administered in experiments of long duration.

Two different samples of β-oestradiol have been used. The first, obtained through the kindness of Dr. E. Oppenheimer, had M.P. = 211°; the second, due to the courtesy of Dr. C. Miescher, had M.P. = 219°**. M.P. of 215 to 224° are given in the bibliography (see Selye, [16]).

Tablets of α-and β-oestradiol were prepared by compression. Rate of absorption from β-oestradiol tablets implanted beneath the skin was found to be slower than that from α-oestradiol tablets. Whereas 1.4 μg per sq.mm and day were absorbed from α-oestradiol tablets (Lipschutz and Vargas, [11]; Fuenzalida, [1]), only about 0.8 μg per sq. mm and day

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* These suggestions were made by doctor Albert Segaloff, Director of Endocrine Research of the Alton Ochsner Medical Foundation, New Orleans, to whom our cordial thanks are due. Dr. Segaloff was also so kind as to read the manuscript.

** Determiniations made by Dr. F. Fuenzalida, Biochemist of the Department.

were absorbed from β-oestradiol tablets. Implantation of ¼ of a tablet up to 5 entire β-oestradiol tablets with a surface of 65 sq. mm each was necessary so as to obtain a range of absorption of 13 to 290 μg of β-oestradiol per day.

Table 1 summarizes the results with 21 animals necropsied 91 to 123 days after subcutaneous implantation of β-oestradiol tablets. Results with 17 animals necropsied 91 to 123 days after subcutaneous implantation of α-oestradiol tablets are given for comparison.

Oestrogenic action was obtained with both samples of β-oestradiol. Considerable increase of uterine weight took place. The genital entrance opened several days after implantation was made and remained so to the end of the experiments. Mammary glands developed, especially with larger quantities of β-oestradiol. As to uterine weights there was no difference between β- and α-oestradiol; increase was with β- indeed more pronounced than with α-oestradiol. However, no significance can be attributed to this divergency on account of the great variation one meets with as to uterine weights in similar experiments.

Microscopical examination of the uterus and vagina was made in 8 animals with an absorption of 13 to 290 μg of β-oestradiol per day, and with uterine weights of less than 1 to 14.5 g. The vaginal mucosa was in full oestrus with cornification in 3 animals receiving 13, 24 and 32 μg of β-oestradiol per day; in the 5 remaining animals with 63 to 290 μg of β-oestradiol per day, the superficial layers of the thick mucosa were undergoing vacuolization, the latter being probably due to a release of antioestrogenic steroids from the adrenal cortex under the influence of oestrogen (see discussion of the phenomenon in ([7]), p. 194). The muscle layers of the large uteri were greatly thickened. Cystic glandular hyperplasia was present in all uteri with the exception of the two smaller ones. Enormous polypous masses filled the cavity of the large uteri. The submucosa was sometimes highly vascularized. The uterine epithelia were cylindric. In experiments lasting 4 months stratification, or pseudostratification, was found. In one of the latter cases invasion of proliferated glands into the circular muscular layer took place.

As to all this there was seemingly no difference between α- and β-oestradiol though α-oestradiol is certainly producing these effects at lower quantitative levels than β-oestradiol. But, as seen from table I, α-and β-oestradiol differed in two other aspects: first, as to the frequency of the genital bleeding, and secondly, as to the degree of the fibrous tumoral effect.

Frequency of bleeding from the genital tract was considerable with β-oestradiol, whereas it was not stated in the present work with α-oestradiol. We must suppose that it has been overlooked in the α-oestradiol-group, because we know from former similar work with α-oestradiol tablets that bleeding occurs (for examples see 7, p. 152). However, in the experiments summarized in table I the animals of the α- and β-oestradiol groups have been examined at equal intervals, and the chance of stating or overlooking the genital bleeding was therefore the same in both.

More consistent is the second difference mentioned: the fact that with β-oestradiol the fibrous tumoral effect, was less pronounced than with α-oestradiol. With 37 to 103 μg of the latter an average of fibrous tumoral effect F.T.E. = 4.6 was obtained within 3 months, and 10 out of 17 animals reached this average. On the contrary, none out of 17 animals with 30 to 290 μg of β-oestradiol had reached the above average. There were in the 17 animals of the α-oestradiol group 25 marks of class 2 and 3, and only 1 mark of class 2 in the 17 animals of the β-oestradiol group. Nothing changed as to this when experiments with β-oestradiol were prolonged for 4 months.

The very weak fibromatogenic action of β-oestradiol is all the more striking as action on the uterus was not less pronounced than with α-oestradiol. This *dissociation* of the two actions, as roughly expressed in uterine weight and in the fibrous tumoral effect, has already been noted for other weak oestrogens, as oestriol, equilenin (Lipschutz, Thibaut and Vargas,[10]) and dihydroequilenin (Mello,[13]). It was the same with derivatives of Miescher's doisynolic acid (Iglesias, Lipschutz and Mardones,[4]), or with very small quantities of α-oestradiol (Riesco Tenorio; see Lipschutz,[7], p. 45, 80).

One may raise the question whether the above results in experiments with the prolonged administration of β-oestradiol could be attributed to small quantities of α-oestradiol possibly present in the β-oestradiol samples used. We are unable to settle this question.

Experiments with the simultaneous administration of α- and β-oestradiol

Table II summarizes the results with 25 castrated female guinea pigs with a subcutaneous α-oestradiol tablet on one side and a β-oestradiol tablet on the other. Necropsy was made 3 months after implantation. For comparison the 17 animals with α-oestradiol tablets and 15 animals with β-oestradiol of table I, of the same duration, also were included in table II.

The first set of experiments (I) was with small quantities of the β-oestradiol sample of Op., the second one (II) with larger quantities of the β-oestradiol sample of Mi.

The difference between the α-oestradiol group and the group with α-and β-oestradiol of the Op. sample was, as to the fibrous tumoral effect, not significant; as shown also by the uterine weight, adding small quantities of β-oestradiol did not change the course of events. With larger quantities of β-oestradiol of the Mi. sample (II) there was seemingly some increase of the fibrous tumoral effect; but the increase was not significant. Variation of the fibrous tumoral effect, as induced by α-oestradiol or other oestrogens is always considerable; it suffices to compare IA and IIA. This is why no importance can be attributed to differences as those between the fibrous tumoral effect in IA and IB, or between the fibrous tumoral effect in IIA and IIB. When larger quantities of β-oestradiol were added, as in IIB, the uterine weight increased in a

TABLE I

Results with the prolonged administration of β-oestradiol to 21 castrated female guinea-pigs, compared to those with the administration of α-oestradiol

Number of anim.	Duration days	Absorb. p. day μg	Bleeding number of anim.	Uterus g	F. T. E. * Average	Number of marks 2 and 3** Average	F. T. E. of each animal of the group †	Sample
		β-oestrad.						
4	91	13-24	0	1.4 (0.95 — 1.8)	0	0	0-0-0-0	Op.
4	91	30-43	0	3.2 (2.0 — 3.7)	0.8 f.	0	0-0-0.5-*2.5*	3 anim. Op.; 1 anim. Mi., 123 d. (Ut. 3.7 g)
2	91-123	63-66	2	14.1 (13.7 — 14.5)	1.0 f.	0	0.5-1.5	Mi.
6	91	92-127	1	6.6 (4.5 — 12.0)	1.3	0	0-0.5-1.5-*1.5*-2-2	Mi., 1 anim. 123 d. (Ut. 5.2 g)
2	91	154-175	1	6.8 (4.0 — 9.5)	1.8	0.5	0.5-3	Mi.
3	123	160-290	1	9.2 (3.5 — 13.0)	1.3	0	*0.5-1-2.5*	Mi.
		α-oestrad.						
4	91-92	37-47	0	4.1 (2.8 — 7.5)	2.7	0.8	1.5-2-2.5-5	
8	91-92	52-65	0	4.4 (3.5 — 6.0)	6.4	2.2	1.5-*4.5*-5.5-5.5-8-8-8-10	
5	91-92	72-103	0	3.3 (2. 8 — 4.1)	3.2	0.8	1.5-1.5-2.5-4.5-6	

* For details see Lipschutz, Vargas, *et al.* [12].
** For details see Lipschutz and Maass, [9].
† Italics: experiments lasting 123 days.
f = only fibrous strands, no tumours.

TABLE II

Experiments with simultaneous administration of α- and β-oestradiol

Group	Number of animals	Duration days	α per day µg	β per day µg	Bleeding number of anim.	Uterus g	F. T.E. * Average	Number of marks 2 and 3* Average	F. T. E. of each animal of the group	Samples of β
IA	8	92	54	0	0	4.6	5.3 ± 1.1	1.6	1.5-2-2.5-5-5.5	—
						(2.8-7.5)			8-8-10	
IB	10	91	76	41	2	4.5	3.8 ± 0.8	1.2	1-1-2-2-2.5-3.5	Op.
				(27-62)		(3.0-8.2)			3.5-7-7-8	
IC	7	91	0	26	0	2.1	0	0	0-0-0-0-0-0-0	Op.
				(13-43)		(0.9-3.5)				
IIA	9	91	51	0	0	3.4	3.9 ± 0.6	1.3	1.5-1.5-1.5-2.5-4.5	—
IIB	15	91	53	87	3	(2.8-4.9)			4.5-5.5-6-8	
				(46-123)		5.9	5.1 ± 0.7	1.5	1.5-1.5-3.5-3.5-3.5-3.5	Mi.
IIC	8	91	0	117	3	(3.0-9.0)			4-4.5-4.5-6-7.5-8-9-11	
				(66-175)		7.7	1.2 †	0.1 ††	0-0.5-0.5-0.5-1.5	Mi.
						(4.0-12.0)			2-2-3 ††	

* See Table I.
† Due to 3 animals receiving 111,127 and 175 µg of β per day with tumors of the uterus and of the spleen.
†† Due to 1 animal receiving 175 µg of β-oestradiol per day, with uterine tumor class 2.

significant manner indeed. Remarkable is also the greater frequency of genital bleeding due to the addition of β-oestradiol.

SUMMARY

Development of mammary glands, cornification of vaginal mucosa, considerable increase of uterine weight, cystic glandulary hyperplasia, with invasive growth of the glands, genital bleeding were produced in the castrated guinea pig with the prolonged administration of sufficient quantities of β-oestradiol absorbed from subcutaneously implanted tablets.

Genital bleeding was with β-oestradiol seemingly more frequent than with α-oestradiol.

Similarly to what has been observed with oestrogenic metahormones as equilenin and dihydroequilenin, with derivatives of the doisynolic acid, or with very small quantities of α-oestradiol, the toxic actions of β-oestradiol on the genital tract were not, or only exceptionally, accompanied by a fibrous tumoral reaction.

β-oestradiol when administered simultaneously with fibromatogenic quantities of α-oestradiol did not influence, or in any way not counteract, the fibromatogenic action of the latter. When larger quantities of β-oestradiol were administered together with α-oestradiol greater uterine weights were obtained without a significant increase of the fibrous tumoral effect taking place.

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THE INTRAPERIOD STRUCTURE OF THE AXIAL REPEATING PERIOD OF THE NEUROTUBULES

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ELECTRON MICROSCOPE analysis revealed a new fibrous constituent in a variety of fragmented nerve fibers (De Robertis and Schmitt, 1948), the axonic nature of which has been conclusively demonstrated in thin sections of medullated nerve fibers and in axons grown in tissue culture (De Robertis, Franchi and Sotelo, 1950). These components were tentatively called neurotubules because the common pattern is that of a fiber of indefinite length having dense edges and a central axis of low density and giving densitometric patterns accross the axis similar to those expected by calculation from a tubular structure (De Robertis, 1950). In addition, after staining a periodic cross banding is generally observed in neurotubules. In human sympathetic and in frog and rabbit sciatic nerves stained with phosphotungstic acid, three bands (α, β and γ) were described. The bands have a typical density, width and position within the period, the α band being most prominent and dense, the β less dense or of about the same density and the γ, very faint and thin (De Robertis and Schmitt, 1948).

Since this work was published, slight modifications in the technique of preparing and staining the material have permitted the resolution of a greater number of bands within the axial period. The intraperiod structure of the neurotubule has been studied from the point of view of the relative position, density and other characteristics of the bands and interband regions.

MATERIAL AND METHODS

Nerve types studied included mainly the sciatic nerve of the rabbit. Some observations were also made on frog and monkey sciatic nerves and other nerve types mentioned in a previous paper. Nerves were fixed in 10 per cent formalin in appropriate Ringer solution, then washed in distilled water and the nerve bundles dissected as free as possible of connective tissue. Fragmentation and preparation of the specimens was

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* Work mainly done at the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

TABLE I

Intraperiod structure of neurotubules from the rabbit sciatic nerve

Specimen Number	Spacing Å	DISTANCES BETWEEN BANDS											
		$\alpha_1 - \alpha_2$		$\alpha_2 - \beta_1$		$\beta_1 - \beta_2$		$\beta_2 - \gamma_1$		$\gamma_1 - \gamma_2$		$\gamma_2 - \alpha_1$	
		%	Å	%	Å	%	Å	%	Å	%	Å	%	Å
1	540	13.6	75	17.1	95	16.2	90	19.6	105	17.3	95	16.2	90
2	550	13.3	70	16.3	90	17.0	95	19.1	105	17.5	95	16.6	95
3	600	13.7	80	16.6	100	16.1	95	19.2	115	17.7	105	16.8	100
4	600	12.9	80	18.2	110	16.7	100	18.9	115	16.9	100	16.5	100
5	600	14.7	90	15.5	95	17.1	100	19.3	115	16.6	100	16.8	100
6	650	14.3	95	15.7	100	16.4	105	19.9	130	17.3	110	16.2	105
7	690	12.9	90	17.8	125	16.9	115	18.5	130	17.7	120	16.3	110
8	910	13.4	120	16.5	150	15.7	145	20.1	190	16.8	155	17.2	155
Average St. Dev.	645	13.6 $\sigma \pm 0.63$	90	16.7 $\sigma \pm 0.94$	110	16.5 $\sigma \pm 0.48$	105	19.3 $\sigma \pm 0.47$	125	17.2 $\sigma \pm 0.41$	110	16.6 $\sigma \pm 0.30$	105
Position of bands in macroperiod.		13.6 %		30.3 %		46.8 %		66.1 %		83 %		100 %	

done as previously described (De Robertis and Schmitt, 1948). Particularly important in revealing a detailed intraperiod structure is the washing of the neurotubules and removal of the amorphous surrounding material. This has been accomplished by repeated high speed centrifugations or by pipetting water on the grid. The staining has been carried out with 0.1 per cent phosphotungstic acid solution at pH 5 before the specimen dries on the grid; then the excess of phosphotungstic acid was washed or blotted off. The duration of staining varied between 30 and 120 seconds. Some specimens were shadowed with 8 mg of chromium at a glancing angle of 6 – 10° and a specimen to filament distance of 20 cm. An R. C. A. type EMB electron microscope was used throughout these studies. The relative position of the bands was established by using a microdensitometer. In each neurotubule the position of the intraperiod bands was obtained by averaging the distances between bands in 10 successive periods. The results were expressed in absolute figures and also in percentages of the total period (table I). In a few electron micrographs of neurotubules microdensitometric tracings along the axis were made so as to estimate the relative density of the bands and interbands. These tracings were compared with records taken from electron micrographs of collagen fibers from hide **.

Results

Figure 3 illustrates, at low magnification, a typical field of neurotubules from a rabbit sciatic nerve. In addition to the thin, dense edges and the amorphous peritubular material, which was washed off to a greater or lesser extent, the neurotubules show the characteristic axial repeating macroperiod. At first glance this period appears to be divided into two segments, one dense and slightly longer, and the other with lower electron density, which alternates regularly along the axis.

The neurotubule in the upper right shows the three bands intraperiod structure previously described (α, β, γ bands) (De Robertis and Schmitt, 1948). In the other four neurotubules 6 and 7 bands can be resolved within the period. In the six bands structures, four bands are found in the dense segment corresponding to the position of the α and β bands and two in the clear region corresponding to the γ band. In the seven bands neurotubules three bands can be resolved in the clear region corresponding to the localization of the former γ band.

Relative position of the bands within the macroperiod. — In fig. 1 the positions of the striations in 3, 6, and 7 bands neurotubules are indicated. In the six banded neurotubules the striations are called α_1, α_2, β_1, β_2, γ_1 and γ_2 to indicate their possible correspondence with the α, β, and γ bands previously described. Table I shows that the relative position of the striations in six bands neurotubules is closely similar in spite of the fact

** Thanks are given to Prof. F. O. Schmitt and Dr. J. Gross, for providing the electron micrographs of collagen from which these tracings were made.

that the total period varies between 540 to 910 Ångstroms. If one assumes that such variations in macroperiod are due to different degrees of stretching or retraction, this would indicate an essentially uniform extensibility of the intraperiod regions.

In the three bands neurotubule the distance α-β is shorter than β-γ and this in turn is slightly longer than the γ-α (fig. 1). In the six bands

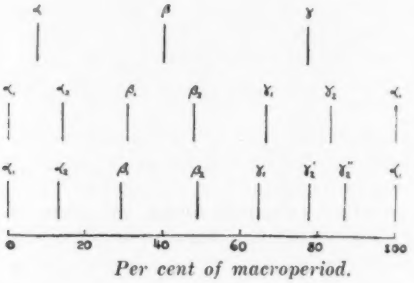

FIG. 1. — *Diagram indicating the relative position of intraperiod bands in three bands, six bands and seven bands neurotubules. The entire macroperiod is represented by 100 per cent.*

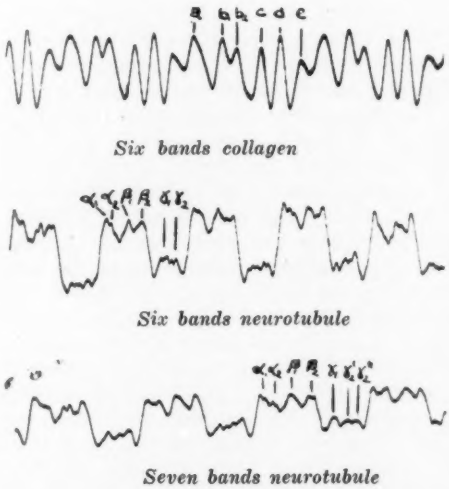

FIG. 2. — *Microdensitometric tracings along the axis of six bands collagen from hide, six bands and seven bands neurotubules. Information can be obtained in relation to the position, width, and relative density of the different intraperiod bands.*

neurotubule (fig. 4) the shortest intraperiod distance is between the α_1 and α_2 bands, while the other distances are almost the same. In the seven bands neurotubule the distance between the sixth and the seventh bands is the shortest. The relative position of these bands suggests that they are related to the γ_2 of the six bands neurotubule (fig. 1). This intraperiod structure is remarkably constant in the repeating periods of individual neurotubules. The particular relative position of the bands inside the period imposes on the tubule an axial polarization throughout its whole length. The actual widths of the bands could only be estimated. Inspection of the electron micrographs and microdensitometer tracings indicates that: β_1 and β_2 are generally wider than α_1; α_2 is rather thin and often difficult to resolve; γ_1 and γ_2 are thin and very faint.

Relative density of the bands. — Microdensitometric tracings along the axis of six and seven bands neurotubules show a characteristic square wave shape which indicates alternating segments of high and low electron density (fig. 2). In the region of high electron density four crests, corresponding to the α_1, α_2, β_1, β_2 bands, are generally well defined. The α_1 peak is higher than the β_1 and β_2; α_2 is the lowest. In the region of low density two or three peaks corresponding to the γ bands can be observed, although sometimes, because of their low density, they are rather obscured by the irregularities of the background. The lowest density is observed in two deep ridges situated between β_2 and γ_1 and γ_2 and α_1.

Study of the intraperiod structure with shadow casting technique. — Three transverse elevations corresponding to the α, β and γ bands were previously described in neurotubules of vertebrate nerves shadowed with chromium (De Robertis and Schmitt, 1948).

Reinvestigation of this problem has revealed that in some neurotubules a more complex intraperiod structure can be observed. The observation of these finer details depends a great deal on the careful washing of the neurotubules so as to eliminate all the surrounding amorphous material. Also the proper orientation of the neurotubule in relation to the direction of the shadowing is important. Shadows making an acute angle with the axis of the neurotubule generally reveal a more detailed structure than do exactly parallel or perpendicular shadows. As shown in figs. 5 and 7, the elevations corresponding to the three main bands are readily visible. In some periods it is possible to see a double elevation in the region of the β and another doublet in the region of the γ band. That gives a total of five elevations (α, β_1, β_2, γ_1, γ_2) per period. It has been impossible to ascertain with certainty the duplication of the α band as in stained preparations.

Discussion

The intraperiod pattern of the neurotubule is of particular significance in the differentiation of this structure from other periodic fibers such as collagen, fibrin, keratin, paramyosin, found in biological materials.

Of these the collagen fibril is the most important because it has a macroperiod essentially identical with that found in neurotubules *** and furthermore, it shows a complex intraperiod structure in which 5, 6 or even 7 bands can be resolved (Schmitt and Gross, 1948). This similarity indicates the possibility that neurotubules may also belong to the "collagen class", postulated by Astbury. However X-ray diffraction data which could prove or disprove this hypothesis are still lacking. Excluding other fundamental differences between collagen and neurotubules statistically significant differences exist between some of the intraperiod spacings of the two materials. The spacing $\alpha_1 - \alpha_2$ (neurotubule) is 28 % larger than a, – b, (collagen) and $\alpha_2 - \beta_1$ is 33 % less than $b_1 - b_2$. However the relative localization of the other bands within the period is quite similar for both materials.

The widths of the bands show only slight differences; while in collagen the α band is the widest, in neurotubules the β_1 and β_2 are wider than the α_1. Furthermore, the γ bands of neurotubules are narrower than any of the striations found in collagen.

The differences in relative density of the bands and interbands are particularly striking. While in unstained preparations neurotubules generally show dense straight edges and a continuous axial core of low density the collagen fibrils show alternating regions of greater and less density with a scalloped edge and highest electron density in the core of the fibril. In preparations of collagen stained with phosphotungstic acid and in which 5 to 7 bands are resolved, the relative density of the various bands show differences which are not as marked as in the neurotubules. Microdensitometric recordings of collagen, along the axis of the fibril, show sharply defined crests and valleys corresponding to the bands and interbands. This is in contrast to similar recordings in neurotubules showing a square shaped scalloping due to the alternating segments of high and low density (fig. 2). In collagen from hide, the *a*, *b*, and *d* bands are approximately of the same density, the b_2, *c* and *e*, being slightly less dense. The *a* band is the most sharply defined. In the neurotubules the γ_1 and γ_2 have a much lower density in comparison to the other bands.

Since the publication of our first paper, more conclusive evidences are available regarding the tubular nature of these structures. Particularly pertinent are the findings of dense particles inside the neurotubules of monkey nerves infected with human poliomyelitis virus (De Robertis and Schmitt, 1949) and B virus (De Robertis, 1949), and the observation of ring shaped cross sections of neurotubules in thin sections of the nerve axon (De Robertis, Franchi and Sotelo, 1950). However, in the intensely stained neurotubules, as those illustrated in this paper, the characteristic tubular appearance is greatly reduced. First glance observation of figures 4 and 5 leads to the impression that they are solid fibers. However,

*** Unpublished observations by Miss W. Van Iterson have shown that, in rabbit sciatic nerve, the macroperiod of the neurotubule is 650 Å, and the distribution varies mainly between 400 and 800 Å. This is in close agreement with the previously published data on the human sympathetic nerve (De Robertis and Schmitt, 1948).

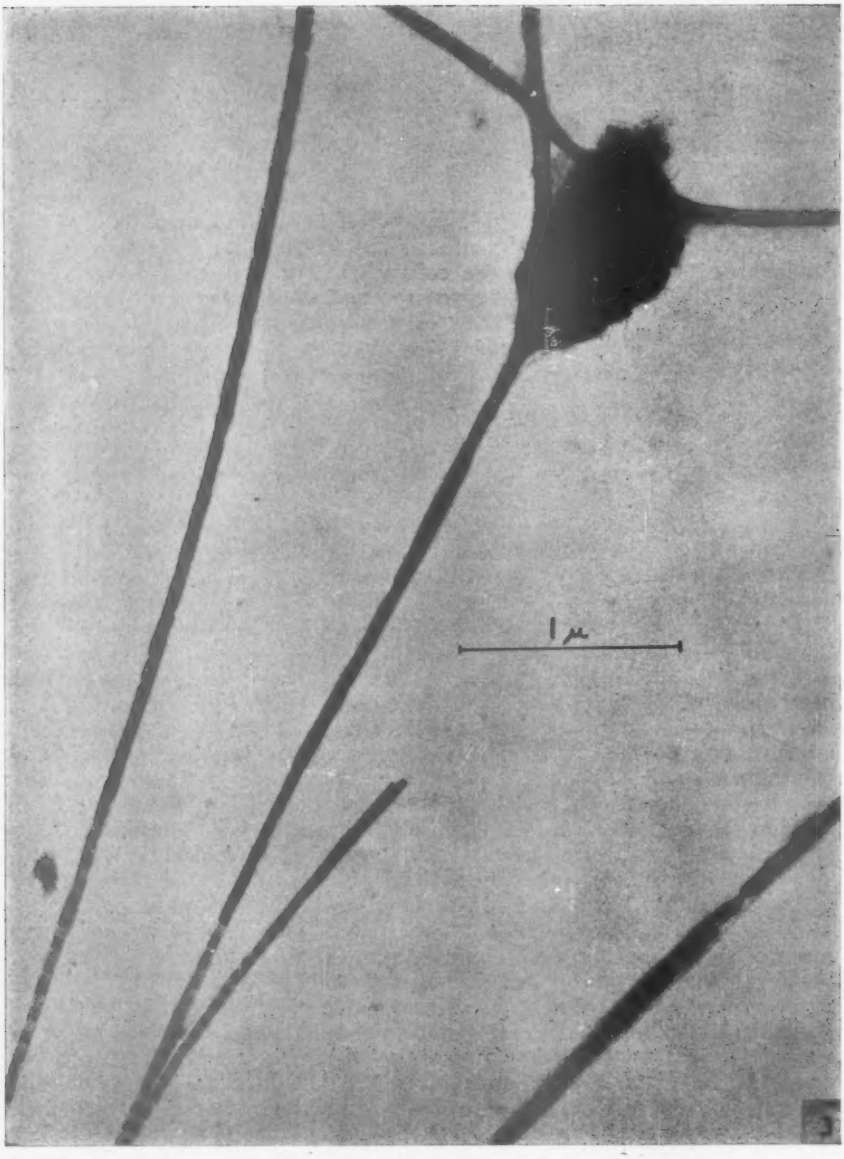

PLATE I

FIG. 3. — *Electron micrographs showing five neurotubules from the rabbit sciatic nerve. The macroperiod is sharply divided into two segments of high and low density. Within the period a number of bands can be resolved: three bands in the upper neurotubule, six bands in the two lower right, and seven bands in the two of the left. Formaldehyde fixation, sonic disintegration, staining with 0.1 % phosphotungstic acid.* × *34 000.*

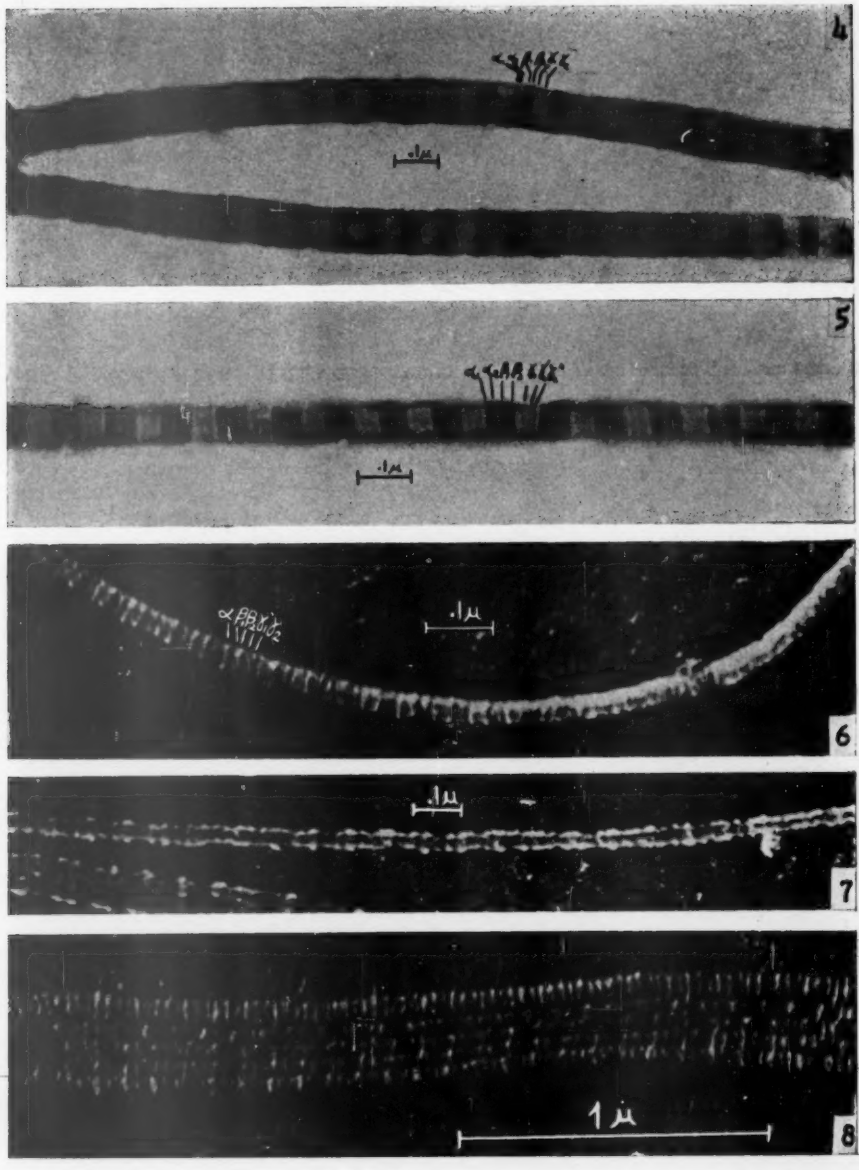

PLATE II. — Electron micrographs of neurotubules of rabbit sciatic nerve, fixed in formaldehyde and disintegrated with sonic oscilations.

FIG. 4. — Two six bands neurotubules. The position, width, and relative density of the bands can be observed. Edges are denser than axis in spite of complete "cleaning" of the neurotubules. Phosphotungstic acid staining. × 68 000.

FIG. 5. — Seven bands neurotubule, phosphotungstic acid staining. × 78 000.

FIG. 6. — Neurotubule shadowed with chromium at an angle of 8°. Five bands can be defined as transverse elevations on the surface × 98 000.

FIG. 7. — Neurotubule shadowed with chromium with the direction of the shadow parallel to the axis. In this particular case, both edges are at a higher level than the core: Periodic striation is also apparent. × 71 000.

FIG. 8. — Bundle of neurotubules shadowed with chromium at an angle of 8°, showing periodic cross banding. × 47 000.

close examination shows in most cases the presence of denser edges. This particular electron density pattern is readily determined by microdensitometric analyses across the neurotubule which shows that the density of the core of the neurotubule is lower than at the edges. Opposite results are obtained on collagen fibrils. As previously described (De Robertis and Schmitt, 1948) the tubular appearance of neurotubules is enhanced by the amorphous dense peritubular material surrounding the neurotubule (fig. 3) but does not depend on it because even after "cleaning" of the neurotubule, the presence of dense edges may be apparent and can be ascertained photometrically. Furthermore, in shadowed preparations when the direction of the shadow is exactly parallel to the axis it is often possible to find that the edges are elevated above the level of the central core (fig. 7). If the concept of the tubular nature of the neurotubule with a continuous core is correct, one has to suppose that the transverse bands are localized at the periphery and represent periodic differentiations of a membrane. It is unknown if the periodic structure represents annular regions differing in actual thickness or in chemical organization or in both. In neurotubules shadowed with chromium the α_1 β_1, β_2, γ_1, γ_2, bands appear as elevated regions on the surface (figs. 6, 7, 8). This would tend to favor the first hypothesis but the possibility of an actual difference in chemical constitution can not be discarded.

Comparing the banded structure shown in this paper with the one previously described (De Robertis and Schmitt, 1948) it is evident that in the latter case, the phosphotungstic acid which was applied after drying the specimen, had penetrated very little and only three thin bands were observed. Staining in wet condition allows more phosphotungstic acid to be fixed in the tubule particularly at the level of the α and β bands. It is impossible for the moment to explain the factors which determine the presence of a 3-6 or 7 bands neurotubules. It is evident that a great deal depends on proper fixation, "cleaning" of the neurotubule, proper staining and proper focussing. All these factors improve the resolution of the electron micrographs. However, in single electron micrographs (fig. 3) it is possible to see neighboring neurotubules having 3, 6 or even 7 bands though some of the above mentioned factors are constant.

The repeating period and the intraperiod structure probably depend on an underlying regularity of configuration or chemical properties of small components in the wall of neurotubules which are now being investigated. The greater or lesser perfection in the alignment of these components may determine the number of intraperiod bands.

Summary

The intraperiod fine structure of the neurotubules of the nerve axon has been analyzed with the electron microscope. Improvement in the technique of preparation and staining of the material permits the resolution of a more detailed intraperiod structure. In neurotubules from the rabbit sciatic nerve in addition to the three intraperiod bands structure previously

described (De Robertis and Schmitt, 1948), six and seven bands have been observed. The relative position, width, and electron density of the bands and interbands within the macroperiod has been studied and microdensitometric tracings along the axons are presented. A fine intraperiod structure with five bands, was determined with shadow casting technique.

Comparison with the intraperiod structure of six bands collagen fibers is discussed, and differences in relative position, width, and density of the bands, are described. The factors which improve resolution of the fine structure are discussed.

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EFFECT OF THYROXIN ON THE OXYGEN CONSUMPTION AND NITROGEN EXCRETION IN ADRENALECTOMIZED RATS

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THE INCREASE in oxygen consumption, which is observed in laboratory animals following the administration of thyroxin, does not occur when adrenalectomy has been performed ([10, 12]). Administration of adrenal cortical extract ([12]) or large doses of desoxycorticosterone acetate (6-7 mg daily, unpublished results) normalized the calorigenic response to thyroxin in adrenalectomized animals. Adrenal cortical hormones thus appear to have a clearcut influence on the capacity of animals to increase their oxidative processes, a view supported by the findings of Tipton *et al.* ([26, 27]) who showed that cortical hormone is involved in the mechanism responsible for the activation of respiratory enzymes in hyperthyroidism.

Considering the relation which exists between energy metabolism and urinary nitrogen excretion, the idea was expressed ([12]) that the high caloric output in hyperthyroidism may be, in part, ascribed to the increased protein utilization and that, on the other hand, the inability of adrenalectomized animals to respond to thyroxin with an increased oxygen consumption could possibly be due to a disturbance in such energy-yielding processes as for example deamination.

The rôle of the adrenal cortex has been elucidated with respect to the variations in nitrogen excretion that occur in animals submitted to stress, such as fasting, fractures, burns, phlorrhizin intoxication, etc. ([29, 30, 3, 13]). In all these conditions, the excretion of nitrogen is lower in the adrenalectomized than in the normal animals. On the other hand, it has been demonstrated that the administration of thyroxin to intact animals leads to an increased nitrogen excretion ([15, 16, 17, 18, 24]).

If we consider the fact that thyroxin is unable to accelerate the oxidative processes of adrenalectomized animals and that, on the other hand, there is evidence that thyroxin modifies the histological aspect ([9, 25]) and the chemical composition of the adrenal cortex (content of glycogenic hormones [2], ascorbic acid [28] and cholesterol [1]), we might reasonably think that, under normal conditions, the adrenal cortex plays an important part in the metabolic changes produced by thyroxin. Little

is known, however, about the nitrogen metabolism of hyperthyroid animals in the absence of the adrenal glands.

The purpose of the present investigation was to study the influence of thyroxin on oxygen consumption and nitrogen excretion in intact and adrenalectomized animals, in order to analyse the relationship which may exist between the capacity for increased calorigenesis and the capacity for protein utilization in the two types of animals.

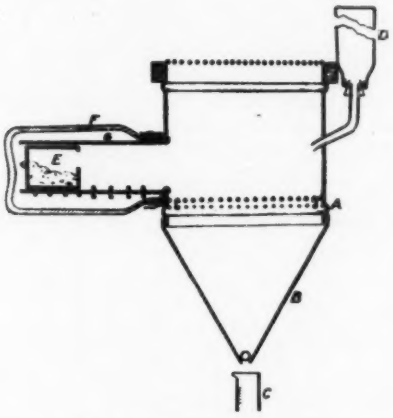

FIG. 1. — Metabolism cage.

METHOD

The experiments were carried out upon rats of both sexes from the animal quarters of the Institute. The rats were kept at room temperature (22–26° C) in special metal metabolism cages which permitted the separate collection of urine and feces, and the measurement, by difference of weight, of the food eaten. In orden to prevent loss of food, and contamination of the urine by alimentary nitrogen, the cages (fig. 1) were provided with specially constructed feeding troughs. Fastened to one wall of each cage was a 0.5 litre screw-topped glass jar F (Ball type) inside which was placed an incomplete metal cylinder G of 5 cm internal diameter and 16 cm long. A longitudinal slot in the wall of this cylinder, 4 cm wide, is placed facing downwards, and is fitted with a series of transverse wire barriers. At the far end of this cylinder is placed the feed box E. By this arrangement, the animal is prevented from dragging food particles to the interior of the cage, the fragments collecting, instead, inside the glass jar. The urine is collected in a bottle C containing 10 % HCl. The feces are retained by a fine wire net placed 2 cm below the coarse wire grid A upon which the animal rests. In some experiments, the diet consisted of a ground commercial feed (the usual diet in

our animal quarters) containing 3.1 % nitrogen; in others, a mixture was used, composed of dried milk powder, starch, sugar, Sherman's salt mixture, cellulose, cod liver oil, and a mixture of water-soluble vitamins. The latter diet contained 2.9 % nitrogen. The drinking water used contained 1 % NaCl. For each of the experimental animals used, daily measurements were made of the body weight, oxygen consumption, and urinary nitrogen excretion. The oxygen consumption was measured during 30 minutes periods in a closed-circuit apparatus (Reignault and Reiset principle) and was expressed in terms of volume consumed per square metre of body surface. The body surface was calculated from the weight, by means of the Benedict formula ([11]). In order to avoid loss of urine during the time that the animals were away from their cages, they were placed in the metabolism apparatus in wire net cylinders provided with a strip of filter paper. The nitrogen excreted, was determined every 24 h. The cages and the filter paper strips were washed carefully with a 10 % solution of HCl, the washings being added to the collected urine, the total volume then being made to 40 ml. The nitrogen determinations were done by the Kjeldahl method, using 1 ml aliquots.

During the preliminary observation period, the animals received *ad libitum* feeding. During the "post-adrenalectomy periods", the sham-operated control animals received amounts of food equivalent to the average quantity that the adrenalectomized animals had taken the day before.

Results

In order to obtain some data about the reactions of simple adrenalectomized and simple hyperthyroid animals, two series of preliminary experiments were carried out.

A. — *Effect of thyroxin on the nitrogen excretion in normal rats.* — In a group of 18 female rats weighing 40 to 50 g each, the average daily nitrogen excretion was 55 to 58 mg, during an observation period of 11 days. At the end of this preliminary period, thyroxin (British Drug Houses) (10 μg/g of body weight daily) was injected into 11 of these animals, and their food intake was restricted to the average value of the controls. Both the nitrogen excretion and the oxygen consumption increased progressively in the treated rats. By the eighteenth day, the oxygen consumption had reached a value double that of the preliminary observation period, and the nitrogen excretion had reached a value of 153 ± 8.5 mg daily. In contrast, the oxygen consumption of the controls on the same day was slightly lower than their initial values, and the nitrogen excretion was 125 ± 3.7 mg daily. The relation between nitrogen excretion and oxygen consumption was similar in both groups, varying between 0.46 and 1.4 mg of nitrogen per litre of oxygen.

B. — *Effect of adrenalectomy on nitrogen excretion.* — 44 female rats were used, weighing between 55 and 75 g each. During a preliminary observation period of 8 days, the average nitrogen excretion varied between limits of 27 and 81 mg daily. Twenty eight of the rats were then adrenalectomized. The nitrogen excretion decreased in both groups, to the same extent, associated with the decreased food intake.

C. — Effect of thyroxin upon the nitrogen excretión in adrenalectomized animals

The experiments were carried out on a group of 18 male rats, and a group of 18 females weighing between 70 and 90 g. During a preliminary 12-day period (period A) the animals received *ad libitum* feeding.

Following this, 13 animals of each group were adrenalectomized. The remaining 5 animals in each group (sham-operated) received the average quantity of food that the adrenalectomized animals had taken (period B). From the second day after the operation, all the animals were treated with thyroxin in daily doses of 10 μg/g (period C). The results of the experiment in the male group are shown in fig. 2, and are similar to those in the females.

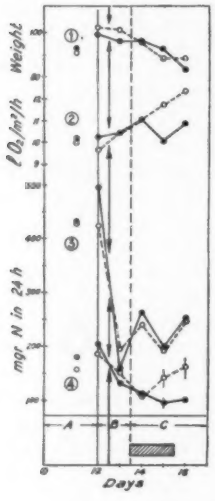

FIG. 2. — Experiment C. 1) Weight, 2) Oxygen consumption, 3) Ingested nitrogen, 4) Excreted nitrogen.
The points in period A show the averages of 11 days. The arrows in period B show the day of the operation. The shadowed area in period C shows the injection of thyroxin.

—— Adrenalectomized
.... Sham-operated.

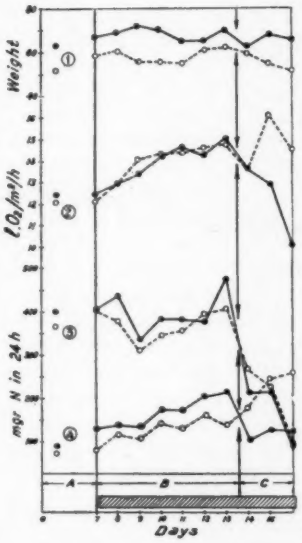

FIG. 3. — Experiment D. 1) Weight, 2) Oxygen consumption, 3) Ingested nitrogen, 4) Excreted nitrogen.
The points in period A show the averages of 6 days. The shadowed area in periods B and C shows the injection of thyroxin. The arrows between period B and C show the day of the operation.

—— Adrenalectomized.
.... Sham-operated.

It can be seen that, during period A, both the ingestion and the excretion of nitrogen, and the oxygen consumption remained at similar levels in both groups of animals. On the first day of period B, the nitrogen intake dropped from about 500 mg to 150 mg, rising slightly during the following days. The oxygen consumption did not change. During period C, the nitrogen excretion decreased progressively in the adrenal-

ectomized animals, and the level of oxygen consumption remained constant, in spite of the continued administration of thyroxin. On the other hand, thyroxin administration in the control animals produced an increase in the nitrogen excretion, proportionate with the oxygen consumption. In the last 2 days of the experiment the standard deviations of the means (s) of the nitrogen excretion of both groups are shown, and it can be seen that the differences are clearly significant ($p = 0.04$).

D. — EFFECT OF ADRENALECTOMY ON RATS TREATED WITH THYROXIN

For these experiments, 13 female rats were used, weighing between 40 and 75 g each. After an observation period of 7 days (period A) all the animals were injected with thyroxin (10 μg/g daily) (period B). At the end of this period, both the oxygen consumption and nitrogen excretion rose about 50 % above the corresponding values during period A. Ten of the animals were then adrenalectomized and three sham-operated, the thyroxin treatment being continued. During this latter period, the food intake of the sham-operated animals which were receiving thyroxin, was restricted to the quantity taken by the adrenalectomized animals.

As shown in fig. 3, the average nitrogen excretion during period A was 88 ± 7.9 mg daily. During the course of period B (thyroxin administration) the nitrogen excretion rose gradually until, by the 6th day of treatment, it had reached an average of 125 ± 7.1 mg. Following the adrenalectomy, the nitrogen excretion dropped sharply in these animals, reaching, on the day following the operation, an average level of 90 ± 10.2 mg. In the control rats (sham-operated) on the other hand, the nitrogen elimination continued to increase, so that by the 15th day of the experiment the average daily value reached 143 ± 2.4 mg, in spite of the fact that the nitrogen intake had been limited in all the animals to an average of 76 mg daily. In contrast, in the adrenalectomized animals, the nitrogen excretion continued to be low, showing an average value of 88 ± 3.2 mg daily, in spite of the continued administration of thyroxin. The differences between the two groups of animals, with respect to the nitrogen excretion, had already become significant on the second day after the operation, and reached a significance of $p = 0.02$ on the third day.

DISCUSSION

It appears to be a well-established fact that protein catabolism is increased in human hyperthyroidism [17] and that there is a significant increase in the urinary nitrogen excretion in animals treated with thyroxin [15, 16, 18, 24]. This effect of thyroxin upon nitrogen is confirmed in our experiments. It seems clear that the increased protein catabolism induced by thyroxin is, in some way, dependent upon adrenal function, since, in the adrenalectomized rats, there was no increase in either the nitrogen excretion or the oxygen consumption. The part of the adrenal gland that is important with respect to this phenomenon, appears to be the cortex, since, as has been shown, the lack of calorigenic response to

thyroxin in adrenalectomized animals is normalized if these animals receive, in addition to thyroxin, an adequate treatment with cortical hormones (12).

The difference in nitrogen excretion between adrenalectomized and normal rats, both receiving equal doses of thyroxin, exists in spite of the food restriction in the control rats. Nevertheless, in our experiments, the possibility of defective intestinal absorption in the adrenalectomized rats cannot be excluded.

Attention has been called to the parallelism between the resting oxygen consumption and nitrogen excretion (7, 23). Similar correlation was also found by Mukkerjee and Mitchell (15) in thyroxin treated rats. These authors established a constant relationship between the creatinine nitrogen output and heat production. Under our experimental conditions, there can be seen clearly a relationship between the oxygen consumption and the total urinary nitrogen. Our results, therefore, seem to show that the oxygen consumption is related to the total protein metabolism and not only to the "endogenous" protein catabolism. Besides, in the light of Schoenheimer's work, there does not appear to be any separation of endogenous and exogenous protein metabolism (5, 6, 21, 22).

The failure to produce an increase in nitrogen excretion and oxygen consumption in adrenalectomized rats, by thyroxin administration, might be explained by an inability of thyroxin, under these circumstances, to accelerate certain tissue enzymatic mechanisms, as has been shown by Tipton *et al.* (27) working with tissue slices from thyroxin-treated rats with adrenal insufficiency. Moreover, deamination appears to be decreased in the absence of adrenal cortical hormones, as has been shown by Russell and Wilhelmi (20) in renal tissue.

Koelsche and Kendall (14) appear to have obtained results that are contrary to ours; but in their experiments a different species was used (dog), and the animals received, in addition to thyroxin, variable quantities of cortical extracts.

The failure of administration of cortical extracts to influence the nitrogen excretion in intact rats treated with thyroxin (4) could be explained if it were accepted that the adrenal glands are already delivering a sufficient amount of hormones. In a similar vein, it is possible to interpret an experiment of Ingle (13) in which he observed that an adrenalectomized rat excreted the same amount of nitrogen as an intact control animal, when both were treated with thyroxin. In this case, it is possible that the adrenalectomized animal had regenerated cortical tissue, which would explain, also, its long survival period (15 days) in spite of thyroxin treatment.

The results of Rupp, Pashkis and Cantarrow (19) showing that the nitrogen excretion is not increased in hypophysectomized rats on thyroxin treatment, might indicate, when taken tokether with our results, that thyroxin activates adrenal cortical function by means of a hypophyseal mechanism.

SUMMARY

1) The administration of thyroxin to normal rats produces an increase in urinary nitrogen excretion, but does not do so in adrenalectomized animals.

2) The increased nitrogen excretion of thyroxin-treated rats diminishes sharply following adrenalectomy.

3) There is a close quantitative relationship between the nitrogen excretion and the oxygen consumption in normal, adrenalectomized, hyperthyroid and hyperthyroid-adrenalectomized rats.

4) Possible mechanisms of action of thyroxin and the adrenal cortical hormones upon protein metabolism are discussed.

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FUNDAMENTAL BASIS OF THE SPECIFICITY OF PRESSOR AND DEPRESSOR AMINES IN THEIR VASCULAR EFFECTS

I. — Blockade of the hypertensive action of vasopressor amines by the effect of the same substances (*)

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THE link of a drug with its cellular receptor has not always been clearv understoodd. After experiments on the formation of antigen-antibodies, Pauling suggested that biological specificity was conducted by the same physico-chemical principles that allow the formation of a crystal ([20]). It has been thought that relations between a certain drug and the cellular receptors, could be explained through Pauling's ideas. Crystallization needs intermolecular surface complementarity; in such a way the molecules can be close enough to be trapped by van der Waal's forces. If this concept is to be applied in Pharmacology, it should be accepted that cellular receptors have a constant morphology and complementary to the molecular surface of the drug.

The so called "pressor amines" and "depressor amines" have the peculiar property of producing tachyphylaxis especially on blood pressure. Although the intimate mechanisms of tachyphylaxis are not clear, there is one hypothesis that is most widely accepted. Such hypothesis indicates that a given drug is bound to the cellular receptor, and stays there for some time (for references, see [23]). According to this, a first dose would occupy a certain number of receptors, a second one occupies some others, and so on. Therefore, after all the receptors are blocked by this occupation, another dose will not produce any action, because there are no more free receptors for the given drug. If compound A can produce hypertension, before compound B has caused tachyphylaxis, and if drug A can not determine hypertension —after tachyphylaxis for drug B is established— it would mean that both drugs occupy the same cellular receptor. In other words, the morphology of a "pressor" or

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"depressor molecule", which induces tachyphylaxis, could be an instrument to explore the cellular receptor for these amines. The extense literature available that correlates the chemical structure of these substances with their pharmacological activity, in relation with the pressor action of Adrenaline, easily allowed this study (for references, see [4]). On the other hand, the inconclusive data dealing with tachyphylaxis induced us to explore this point further.

If Pauling's concepts are to be applied, not only surface complementarity, but mechanisms that control stability of the specifically adsorbed molecule must be analyzed to understand tachyphylaxis. On the other hand, hypertension caused by "pressor amines", would be an algebraic summation of the statistical probabilities that these molecules have to be anchored to specific receptors, localized in the vascular territory. There would be one specific receptor for hypertensive phenomena and others for hypotensive phenomena ([7]). For this theory to be valid, it is interesting to verify the following: if a molecule induces tachyphylaxis through this mechanism, it must block the effects caused by any other molecule with less affinity for the receptor, even if it does not produce tachyphylaxis. Inversely, tachyphylaxis produced by a drug must disappear after the action of other molecules of greater complementarity but of less stability.

In order to study these points, 24 aromatic monoamines were studied (21 hypertensive and 3 hypotensive). Blood pressure in the cat was used as a test, because of the simple technique which easily allows comparative studies.

METHODS

Animals were anaesthetized with a solution of sodium pentobarbital-urethane (urethane, 25 % solution in 3.3 % solution of nembutal "Abbott"). Two hundred and twenty five cats were used throughout the experiments, anaesthetized with 1 cm^3 per kg of this solution, intraperitoneally. Blood pressure levels were measured by connecting the carotid artery to a mercury manometer. Both vagus were cut at the neck. The animal was atropinized with 1-1.5 mg (atropine sulphate "Merck") per kg. A tracheal cannula was placed if artificial respiration was necessary. The drugs used and their concentration were the following:

(1) Isuprel sulphate at 0.5 %.
(2) Butanefrine hydrochloride at 0.5 %.
(3) Arterenol bitartrate monhydrate at 0.1 %.
(4) Epinine hydrochloride at 0.1 %.
(5) Cobefrine hydrochloride at 0.1 %.
(6) Adrenaline hydrochloride (commercial solution of Adrenaline "Clin" at 0.1 %; also Adrenaline F.E.U. of S. B. Penick & Co. was used).
(7) Neo-synephrine hydrochloride at 0.1 %.
(8) Tyramine hydrochloride (Eastman Kodak Co.) at 1 %.
(9) Paredrine bromhydrate at 0.2 %.

(10) Phenylethylamine hydrochloride (Hoffmann La-Roche) at 1 %.
(11) Phenyl-n-propylamine sulphate at 0.5 %.
(12) Phenylbutylamine hydrochloride at 1 %.
(13) Vonedrine hydrochloride at 0.5 %.
(14) Propadrine base at 0.5 %.
(15) Ephedrine hydrochloride (in some instances Ephetonine "Merck" was used) at 1 %.
(16) Metoxamine hydrochloride at 1 %.
(17) β (2,5-dimetoxyphenyl)-β-hydroxyethylamine at 1 %.
(18) Amphetamine sulphate at 0.5 %.
(19) 1-cyclohexyl-2-propylamine at 1 %.
(20) 2-methylamino-1-cyclopentylpropane at 1 %.
(21) Tuamine sulphate at 1 %.
(22) Fouramine at 1 %.
(23) 2-isopropylamine-1-(3,4-dihydroxyphenyl)-butanol at 0.5 %.
(24) Isopropylamine sulphate at 20 % *.

Chemical structures of these compounds and used doses appear in table I. Other drugs employed were: 1 mg of barium chloride ("Merck"), Pitressin (Pituitrol fuerte "Sanitas") in doses of 2 U, Hypertensin in doses of 2 U., and Privine "Ciba" (2-naphtil-imidazoline) in doses from 1 mg to 2 mg. Drugs were dissolved in distilled water and injected in the femoral vein in a volume from 0.1 ml to 0.2 ml. If drugs caused tachyphylaxis and it was necessary to reinject the same drug, this was done after hypertensive action was stabilized or completely gone. This is the reason why time between injection have considerable variation (generally these times were progressively shorter). To insure preservation of the drugs in solution, a drop of 1 % Merthiolate tincture (Eli Lilly & Co.), was added to 20 ml of the solutions. Solutions of Adrenaline, Cobefrine, Epinine, Arterenol, 2-isopropyl-amine—(3,4-dihydroxyphenyl)-butanol, Neo-Synephrine, Butanefrine and Isuprel, prepared from a stock solution were diluted immediately before the experiment with distilled water.

To stabilize stock solution of Adrenaline, Cobefrine, Epinine, Arterenol, Neo-Synephrine, Butanephrine, Isuprel and 2-isopropylamine-1-(2,4-dihydroxyphenyl)—butanol, sodium bisulphite "Merck" was added at the necessary concentration to reach a value of 0.1 %. Merthiolate and sodium bisulphite did not alter the results obtained.

* Drugs 15 and 18 are of unknown trade mark (both were bought in a pharmacy).

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Without these generous gifts, this work would not have been possible. All the employed substances are racemic, except Arterenol which levogyrate.

RESULTS

I. — Amines of bencenic ring

To this group belong a number of compounds that induce tachyphylaxis with more or less facility (table I). Amphetamine has been studied more extensively because it is a compound that induces tachyphylaxis more easily than other of the same group.

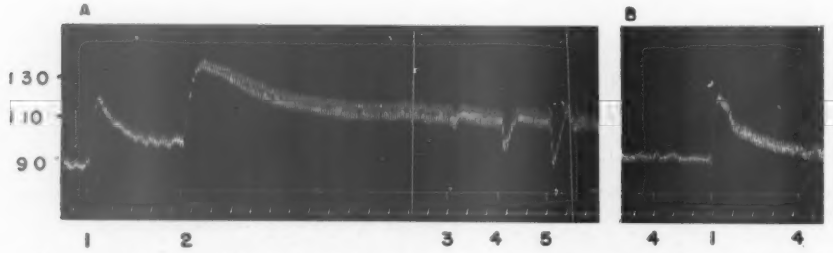


FIG. 1. — *Amphetaminic tachyphylaxis both for hypertensive and hypotensive action. Hypotension is proportional to the injected dose. Amphetamine does not block the hypertensive effect of Neo-Synephrine. 1 800 g cat anaesthetized with sodium pentobarbital.*

Between A and B, 39 minutes and 14 injections of 2.5 mg of Amphetamine. Between both records there is an hipotension of 10 mm Hg. In this and the other figures drugs were injected intravenously at the signals. Lower trace, time in minutes.

1) 20 micrograms of Neo-Synephrine. 2) 2 mg of Amphetamine. 3) 0.5 mg of Amphetamine. 4) 0.5 mg of Amphetamine.

1) *Amphetamine.* — As is well known, if tachyphylaxis has been obtained after an injection of Amphetamine, a new dose of this substance, either causes no effect upon blood pressure, or produces a slight hypotension. Under this condition, injections of larger doses, determine proportional hypotensions (fig. 1). A lethal hypotensive effect can be obtained with considerably larger doses. Tachyphylaxis can also be obtained for hypotension produced by the substance (fig. 1).

Statistical analysis show (see discussion) the possibility that single doses of Amphetamine do cause complete tachyphylaxis. When this phenomenon was induced by this dose, administration of bencenic and cyclic ring amines did not produce hypertension, i.e., crossed tachyphylaxis was obtained for these substances. Similar facts have already been described ([6]). A different behavior was observed for amines of dimethoxyphenilic ring, because these agents can induce hypertension after tachyphylaxis for Amphetamine was completed (see fig. 5, and "amines of dimethoxyphenylic ring"). The blockade of cellular receptors caused by Amphetamine may also be seen for other pressor amines, like those which have a phenolic structure. They can inhibit the effects of Paredrine; to a lesser degree the hypertensive effect of Tyramine (fig. 2) and

they have no action on hypertension produced by Neo-Synephrine (figs. 1 and 2).

As it is known, repeated doses of Amphetamine reduce hypertension produced by Adrenaline. Hypertension caused by Cobefrine and Arterenol may be diminished also by repeated doses of Amphetamine. In all these cases, Amphetamine doses were far above the necessary minimum to occupy the cellular receptor. Nevertheless, in such case, Privine, hypertensin, Pitressin and barium chloride cause an increase in blood pressure comparable to that obtained before Amphetamine injections.

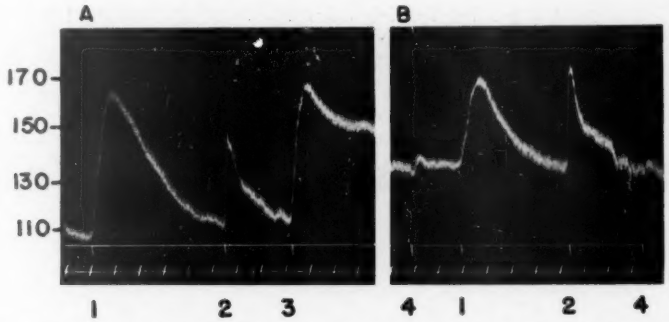


FIG. 2. — *Partial amphetaminic blockade of Tyramine, and Amphetamine failure to block Neo-Synephrine responses. 2 600 g cat, anaesthetized with sodium pentobarbital.*

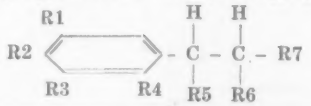
Between A and B, 16 minutes and 3 injections of 0.5 mg of Amphetamine. 1) 1 mg of Tyramine. 2) 20 micrograms of Neo-Synephrine. 3) 2.5 mg of Amphetamine. 4) 0.5 mg of Amphetamine.

2) *Ephedrine.* — This drug also causes tachyphylaxis without difficulty (see table I). In some cases it may produce hypotension, that has not been found by some authors ([24]). On certain opportunities, to obtain hypotension, it was necessary to inject larger doses than those usually needed to induce tachyphylaxis (see table I). When tachyphylaxis to Ephedrine was present, later injections of Amphetamine, Paredrine, Phenylethylamine or Phenylpropylamine did not change blood pressure values. Also, it was observed that Paredrine effects were reduced; (to a lesser extent Tyramine actions and even less the effects of Neo-Synephrine). Larger doses than those needed to occupy all the receptors were necessary to obtain these results. If the ammount is even larger, Adrenaline, Cobefrine and Arterenol may reduce hypertension to lower values than those obtained before Ephedrine. On the other hand, hypertension caused by barium chloride, hypertensin and Pitressin is not modified.

3) *Propadrine and Phenyl-n-propylamine.* — Tachyphylaxis caused by these compounds is more difficult to obtain than that produced by Ephedrine (see table I) ([6]). These substances were less studied because

TABLE I

Chemical structure, doses, degree of hypertension and of tachyphylaxis of the employed drugs.



	R1	R2	R3	R4	R5	R6	R7	Doses mg	Hypertension mm Hg (1)	Tach. (*)
Isuprel		OH	OH		OH	NH-CH-(CH3)2	H	0.50	— 30 ± 0.67 (6)	10.2
Butanefrine		OH	OH		OH	NH2	CH2-CH3	0.50	— 17 ± 1.42 (7)	1.0
Win 3046 (9)		OH	OH		OH	NH-CH-(CH3)2	CH2-CH3	0.50	— 19 ± 1.70 (9)	9.0
Arterenol		OH	OH		OH	NH2	H	0.01	+ 31 ± 2.19 (11)	
Epinine		OH	OH		H	NH-CH3	H	0.04	+ 15 ± 2.00 (8)	
Cobefrine		OH	OH		OH	NH2	CH3	0.04	+ 26 ± 1.26 (10)	
Adrenaline		OH	OH		OH	NH-CH3	H	0.01	+ 22 ± 1.61 (10)	
Neo-Synephrine ..			OH		OH	NH-CH3	H	0.02	+ 17 ± 1.22 (16)	
Tyramine		OH			H	NH2	H	1.00	+ 26 ± 1.64 (15)	42.2
Paredrine		OH			H	NH2	CH3	0.40	+ 19 ± 2.86 (8)	7.75
P. E. A. (9)					H	NH2	H	1.00	+ 21 ± 3.31 (6)	45.3
P. P. A. (9)					CH2	NH2	H	1.00	± 19 ± 2.60 (5)	23.3
P. B. A. (9)					H	NH2	CH2-CH3	1.00	+ 15 ± 2.10 (9)	3.66
Vonedrine					CH2	NH-CH3	H	1.00	+ 20 ± 3.50 (7)	16.0
Propadrine					OH	NH2	CH3	1.00	+ 16 ± 2.00 (6)	24.0
Ephedrine					OH	NH-CH3	CH3	1.00	+ 20 ± 1.91 (8)	7.62
Amphetamine					H	NH2	CH3	0.50	+ 20 ± 1.92 (16)	3.42
Metoxamine	CH3O			CH3O	OH	NH2	CH3	0.50	x	x
832 (9)	CH3O			CH3O	OH	NH2	H	1.00	x	x
S. K. F. 10 cyclohexyl					H	NH2	CH3	1.00	+ 16 ± 1.84 (7)	3.50
cyclopentyl					H	NH-CH3	CH3	1.00	+ 25 ± 3.00 (7)	3 x
Tuamine (2-amine-heptane)								1.00	+ 18 ± 2.60 (6)	6.30
Fouramine (2-amine-4-methylhexane)								1.00	+ 18 ± 2.80 (6)	5.50
Isopropylamine								40.00	x	x

(1) Sign-before numbers means hypotension; sign +, hypertension. Parenthesis, number of animals used to calculate Standard Error: $\sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$

(9) Win 3046, 2-isopropylamine-1-(3,4-dihydroxyphenyl)-butanol. P. E. A., Phenylethylamine. P. P. A., Phenyl-n-propylamine. P. B. A., Phenylbutylamine. 832, β-(2,5-dimethoxyphenyl)-β-hydroxyethylamine. S. K. F. 10, 1-cyclohexyl-2-propyllamine.

(*) See text.

they do not produce tachyphylaxis with facility. Nevertheless, Propadrine can block the hypertensive effects of Ephedrine, Amphetamine, Phenylethylamine and Phenylpropylamine. After tachyphylaxis induced by Phenylpropylamine, the hypertensive actions of Amphetamine and Propadrine were blocked. Once that tachyphylaxis for Propadrine and Phenylpropylamine was obtained, other doses of the same drugs may cause slight hypotension.

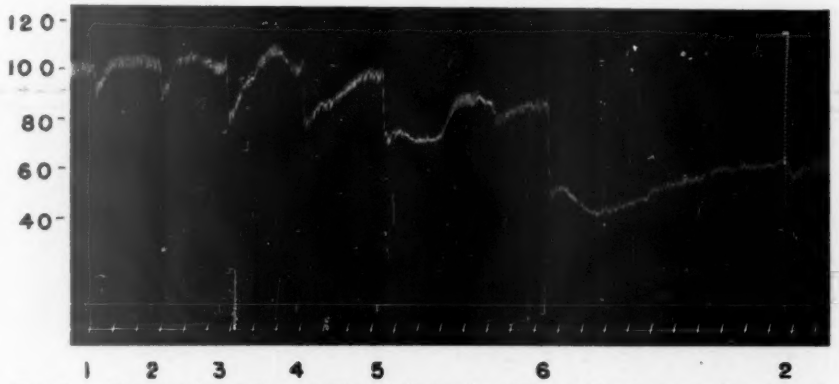


FIG. 3. — *Hypotensive action of Phenylethylamine. This action is proportional to the injected dose. Tachyphylaxis to the hypotensive action is quite small. 2 400 g cat, anaesthetized with sodium pentobarbital.*
1) 1 mg, 2) 2.2 mg, 3) 3.5 mg, 4) 7.5 mg, 5) 10 mg and 6) 27.5 mg of Phenylethylamine.

4) *Phenylethylamine.* — Crismon and Tainter ([6]) observed that this drug easily caused tachyphylaxis. Our results do not agree with this observation because after a great number of single doses of 1 mg (13 or more injections) it produces hypertension 30 % lower than for the first injection. But, if other amines of bencenic ring are previously injected —at doses at which they produce tachyphylaxis— Phenylethylamine does not cause hypertension but hypotension, proportionally to the injected dose (fig. 3). If the dose is too large, a lethal hypotension is observed. As it can be seen in fig. 3, it is very difficult to determine tachyphylaxis due to the hypotensive effect of this drug.

5) *Phenylbutylamine.* — It is an hypertensive drug, but repeated injections finally cause hypotension ([16] and table I). When this phenomenon is established. Vonedrine, Amphetamine, 1-cyclohexyl-2-propylamine, 2-methylamine-1-cyclopentylpropane, Tuamine and Fouramine, either produce no increase in blood pressure or they are hypotensive. After establishment of tachyphylaxis for Phenylbutylamine and Vonedrine, dimethoxyphenylic ring amines cause marked hypertension.

II. — Amines of cyclic ring

1) 2-*methylamine*-1-*cyclopentylpropane.* — This drug produces tachyphylaxis ([22]) with great facility (table I). When tachyphylaxis was obtained, administration of other doses cause an important hypotension, proportional to the given doses (fig. 4). This drug was the most hypotensive one —among the studied— if an equipressor dose was used. In some animals, once hypertension vanished by successive injections of the same substance, hypotension appeared, followed by hypertension (fig. 4). Because of this modality —absent for other substances— it is very diffi-

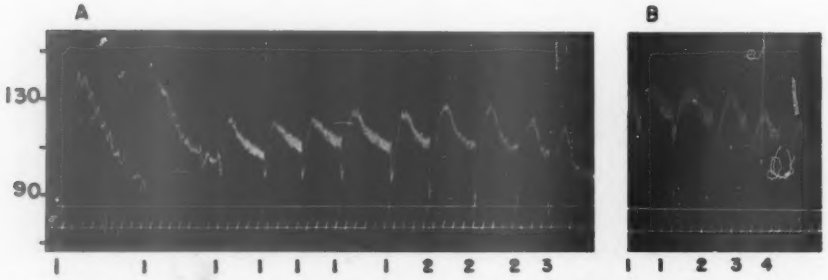


FIG. 4. — *Tachyphylaxis to the hypertensive action of 2-methylamine-1-cyclopentylpropane. The hypotensive effect of this drug is proportional to the administered dose.*
A: 1 800 g cat anaesthetized with sodium pentobarbital.
1) 1 mg, 2) 2 mg, 3) 3.3 mg of 2-methylamine-1-cyclopentylpropane.
B: 2 400 g cat, anaesthetized with sodium pentobarbital. Seven doses of 2-methylamine-1-cyclopentylpropane were previously injected in 39 minutes.
1) 1 mg, 2) 3 mg, 3) 4.5 mg and 4) 5 mg of the drug.

cult to evaluate the exact moment when tachyphylaxis was induced (see discussion). In contrast to amphetaminic and phenylethylaminic responses, this drug does not produce tachyphylaxis for hypotension. If the hypertensive cellular receptor was blocked by 2-methylamine-1-cyclopentylpropane, then Phenylethylamine, Vonedrine, Amphetamine, 1-cyclohexyl-2-propylamine, Tuamine, Fouramine do not modify blood pressure or they cause hypotension.

2) 1-*cyclohexyl* 2-*propylamine.* — The action of this substance is similar to Amphetamine. It rapidly induces tachyphylaxis (table I). Under this condition another dose causes some hypotension, and it blocks the hypertensive actions of Phenylbutylamine, Amphetamine, 2-methylamine-1-cyclopentylpropane, Tuamine or Fouramine, or induces them to determine hypotension (fig. 5). Nevertheless, amines of dimethoxyphenylic structure may cause an important hypertension (fig. 5). Some

authors have not observed tachyphylaxis for this compound, but this is probably due to long spaced injections (14).

III. — Amines of dimethoxyphenylic ring

1) *Metoxamine and β-(2,5-dimethoxyphenyl)-β-hydroxyethylamine.* — These are substances of similar action. They cause hypertension in which the nervous system plays an important role (9). Both in the animal without nervous system (Elliott's preparation) or in an intact animal, serial injections produce tachyphylaxis (8). Nevertheless, tachyphylaxis is more rapidly induced in animals under Elliott's preparation (9). If one of these drugs is injected in an animal without CNS, it may cause tachy-

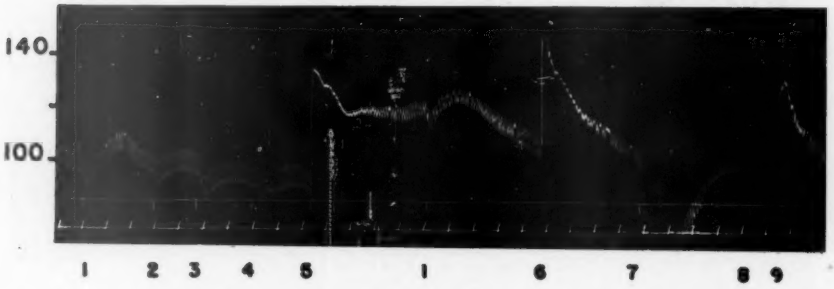


FIG. 5. — *Tachyphylaxis to the hypertensive action of 1-cyclohexyl-2-propylamine and blockade of the pressor effects of other "pressor amines". Hypertensión after amines of dimetoxyphenylic ring is not blocked. 2 000 g cat anaesthetized with sodium pentobarbital.*

Seven doses of 1-cyclohexyl-2-propylamine were injected previously, in 42 minutes.

1) 1 mg of cyclohexyl-2-propylamine. 2) 0.5 mg of Amphetamine. 3) 1 mg of Phenylbutylamine. 4) 1 mg of Vonedrine. 5) 0.5 mg of Metoxamine. 6) 1 mg of β-(2,5-dimetoxyphenyl)-β-hydroxyethylamine. 7) 10 mg of 2-methylamine-1-cyclopentylpropane. 8) 1 mg of Fouramine and 9) 0.5 mg of Metoxamine.

not modify blood pressure or induces slight hypotension. Under this condition Amphetamine either does not affect blood pressure or pressure may fall. On the other hand, if intact animals are used, and tachyphylaxis has been induced by a previous injection of bencenic and cyclic ring, or aliphatic chain amines, Metoxamine and β-(2,5-dimetoxyphenyl) β-hydroxyethylamine, cause an important hypertension (fig. 5). Due to the intense action on the nervous system, these drugs were neither statistically studied on their hypertensive actions, nor their ability to induce tachyhylaxis was considered. The action of dimethoxyphenylic structures on the nervous system is much more marked than the effect of other amines on it.

IV. — Pressor amines of phenolic ring

In this group of "pressor amines", Paredrine, Tyramine, and Neo-Synephrine were studied. Paredrine was selectively studied because of the facility with which induces tachyphylaxis (table I). This agrees with other observations ([6]).

1) *Paredrine.* — After tachyphylaxis was obtained, it was not possible to observe hypotension, even with injections larger than usual (see table I). Under Paredrine tachyphylaxis the administration of Amphetamine, Ephedrine, Phenylethylamine, Phenylpropylamine and Propadrine,

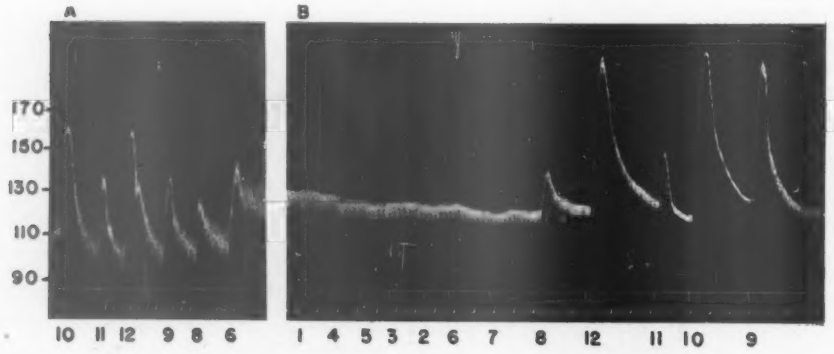


FIG. 6. — *Total Paredrine blockade of the action of bencenic and phenolic ring "pressor amines" (except Neo-Synephrine). Paredrine does not block the hypertensive effects of catecholic ring amines. 2 200 g cat anaesthetized with sodium pentobarbital.*

Between A and B, 58 minutes and 8 injections of 0.4 mg of Paredrine.

10) 40 mg of Cobefrine. 11) 40 micrograms of Epinine. 12) 10 micrograms of Arterenol. 9) 10 micrograms of Adrenaline. 8) 20 micrograms of Neo-Synephrine. 6) 0.4 mg of Paredrine. 1) 0.5 mg of Amphetamine. 2) 1 mg of Ephedrine. 3) 1 mg of Propadrine. 4) 1 mg of Phenylethylamine. 5) 1 mg of Phenylpropylamine and 7) 1 mg of Propadrine.

in different experiments, does not produce hypertension (fig. 6). This drug also may reduce or even obliterate the effect of Tyramine (fig. 6). Hypertension after Neo-Synephrine may also diminish considerably (fig. 7). It may reduce hypertension produced by Adrenaline, Cobefrine and Arterenol if many previous injections of the substance have been given. If this condition is not present, amines of catecholic structure do not alter its hypertensive action (fig. 6).

2) *Tyramine and Neo-Synephrine.* — Practically they do not cause marked tachyphylaxis (table I) ([6]). After 10, 15 or more injections of 1 mg of Tyramine, its hypertensive response was reduced only about

30 %. Under these conditions, the animal may give very good hypertensive responses after the injection of any of the drugs analyzed in this work. Tachyphylaxis after Neo-Synephrine is even less marked, so, it may be considered a non-tachyphylactic drug at the usually employed doses. The injection of other substances produces hypertension after many successive doses of Neo-Synephrine.

V. — Animes of catecholic ring

Two types of drugs were used: "pressor amines" and "depressor amines". To the first group belong Adrenaline, Cobefrine, Epinine and Arterenol. To the second group belong Butanefrine, Isuprel and 2-isopropylamine-1 (3,4-dihydroxyphenyl)-butanol.

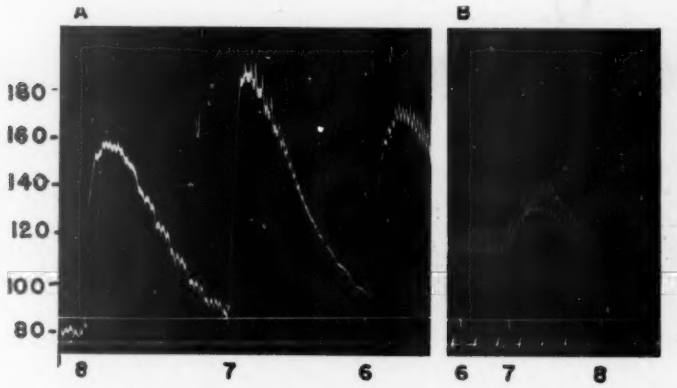


FIG. 7. — *Tachyphylaxis to the hypertensive action of Paredrine. Partial blockade of Tyramine and Neo-Synephrine actions by Paredrine. 2 830 g cat anaesthetized with sodium pentobarbital.*
Between A and B, 49 minutes and 11 injections of 0.4 mg of Paredrine.
8) 20 micrograms of Neo-Synephrine. 7) 1 mg of Tyramine. 6) 0.4 mg of Paredrine.

A) *Hypertensive substances.* — The hypertensive drugs were studied in order to compare their effects with hypertension present after tachyphylaxis was induced by drugs studied in other sections. Results obtained are indicated in table I. As is well known, these substances do not cause tachyphylaxis at the indicated doses ([6]).

B) *Hypotensive substances.* — 1) Butanefrine and Isuprel. These two drugs act in a similar way. After some injections, hypotension becomes hypertension ([5, 12]). This phenomenon appears more rapidly if Butanefrine is used. Butanefrine hypertension is higher than Isuprel hypertension (fig. 10). Once hypotension after Butanefrine has become hypertension, further injections of the drug induce tachyphylaxis for hyperten-

sion (fig. 8). During this stage, administration of other "pressor amines" gave the following results: the hypertensive action of Amphetamine was reversed (fig. 8), the hypertensive responses to Tyramine disappeared (fig. 8), a slight hypertensive response to Neo-Synephrine (fig. 8), considerable reduction of Cobefrine, Epinine and Arterenol effects. On the other hand, Adrenaline may be even more hypertensive than before Butanefrine administration (fig. 8), or it may reduce its hypertensive responses. If Butanephrine hypotensive response is transformed in hypertension, administration of Isuprel always causes hypotension, qualitatively similar to that obtained in animals that have not received any drug (fig. 8). Isuprel behaves like Butanefrine, but, as a blocking agent of

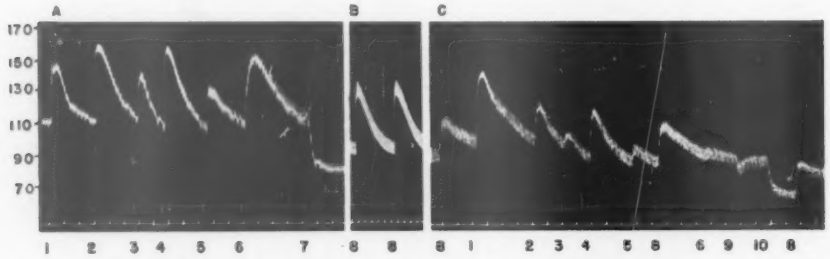


FIG. 8. — *Complete tachyphylaxis to the hypotensive action of Butanephrine and partial tachyphylaxis to the hypertensive action. Complete blockade of the hypertensive action of Tyramine and Amphetamine and partial blockade of the pressor action of "pressor amines" of catecholic structure. 2 800 g cat anaesthetized with sodium pentobarbital.*

Between A and B: 22 minutes and 2 injections of 0.5 mg of Butanefrine, and later 2 injections of 1.5 mg of the same drug.

Between B and C: 56 minutes, 6 injections of 1.5 mg of Butanefrine. Later, 2.5 mg of the same drug injected five times, and then one injection of 1.5 mg of the substance.

1) 10 micrograms of Adrenaline. 2) 40 micrograms of Cobefrine. 3) 40 micrograms of Epinine. 4) 10 micrograms of Arterenol. 5) 20 micrograms of Neo-Synephrine. 6) 1 mg of Tyramine. 7) 0.5 mg of Butanefrine. 8) 1.5 mg of Butanefrine. 9) 0.5 mg of Amphetamine, and 10) 0.5 mg of Isuprel.

the hypertensive effects of "pressor amines" is less effective. If Butanefrine is injected after repeated doses of Isuprel, it determines hypertension, even if given before the total disappearence of the hypertensive action of Isuprel.

2) 2-isopropylamine-1 (3,4-dihydroxyphenyl)-butanol (for simplicity this drug will be mentioned as Win 3046, according to Winthrop's terminology). This substance has a very similar action to the other hypotensive drugs, already studied. At low doses (5 micrograms) it is more hypotensive than Butanefrine but less than Isuprel. This agrees with data indicated in the literature ([13]). Injecting 0.5 mg a prolonged hypotension is obtained, of longer duration than for the other hypotensive substances. Repeated doses of 0.5 mg induces tachyphylaxis for hypotension (fig. 9). Tachyphylaxis por Win 3046 is intermediate —in what facility is concerned—

between Butanefrine and Isuprel; this drug is closer to Isuprel than to Butanefrine (table I). Once tachyphylaxis for Win 3046 is established, Butanefrine administration gives hypertension; in such a condition Isuprel induces slight hypotension. When several doses of Win 3046 are previously administered, Isuprel does not modify blood pressure or

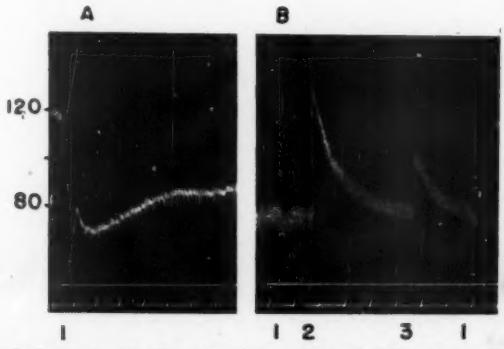


FIG. 9. — *Tachyphylaxis to the hypotensive action of Win 3046. Once tachyphylaxis to the hypotensive action of Win 3046 has been induced, Adrenaline is more hypertensive than Arterenol. 3 150 g cat anaesthetized with sodium pentobarbital.*

Betwen A and B: 46 minutes and 11 injections of 0.5 mg of Win 3046.

1) 0.5 mg of Win 3046. 2) 10 micrograms of Adrenaline and 3) 10 micrograms of Arterenol.

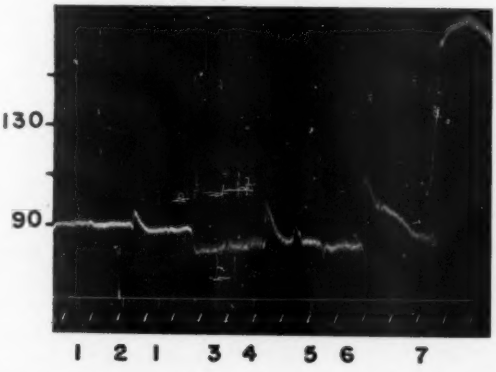


FIG. 10. — *Hypertensive actions of Win 3046, Isuprel and Butanefrine, once the hypotensive receptor has been blocked by previous injections of Win 3046. 2 350 g cat anaesthetized with sodium pentobarbital.*

A previous state of tachyphylaxis was obtained after 11 injections of 0.5 mg of Win 3046 in 42 minutes.

1) 0.5 mg of Win 3046. 2) 0.5 mg of Isuprel. 3) 2 mg of Win 3046. 4) 2 mg of Isuprel. 5) 4 mg of Win 3046. 6) 4 mg of Isuprel and 7) 4 mg of Butanefrine.

induces small hypertension. Inversely, after Isuprel has determined tachyphylaxis for hypotension, Win 3046 does not change blood pressure. If tachyphylaxis for hypotension has been established (after Win 3046) further injections of the drug do not cause hypertension, even after doses eight times larger than usual (fig. 10). Equal doses of Isuprel and Butanefrine, under the same conditions, determine an important hypertension, especially the latter one (fig. 10).

Discussion

"Pressor amines" may modify blood pressure through several mechanisms. Most of pressure regulating reflexes have been eliminated after section of the cervical vagus and by the action of atropine. The hypertensive mechanism may not be greatly influenced by tachycardia; after the use of Dibenamine (N,N-dibenzyl-chloroethylamine) that blocks adrenergic effects without modifying the heart beat ([19]) hypertension induced by "pressor amines" disappears or is considerably reduced ([10]). Nervous system influences may also be of little importance. This is emphasized considering that some drugs —as it is known— cause great stimulation of the nervous system, while others do not, and their actions are comparable at the employed doses. According to our results, that confirm the working hypothesis, the stimulating effects on the ganglion and post-synaptic sympathetic fibers may be negligible. Therefore, we think that "pressor amines" induce hypertension especially by direct action on the smooth muscle fibres of blood vessels. The whole work refers to this direct action.

Our results indicate that amines of bencenic structure (Amphetamine was especially studied) may occupy the hypertensive cellular receptor; in this way they block responses of other "pressor amines" with the exception of catecholic ring amines. On the other hand, hypotensive catecholic ring amines, after determinig tachyphylaxis for hypotension —when doses have been repeated several times— are able to block the hypertensive responses of "pressor amines" (amines of catecholic ring, phenolic ring, cyclic ring and of aliphatic chain). Considering these facts together with data available in the literature ([11]) it is possible to think that a hypertensive receptor of specific morphology is present in the vascular territory. This specific receptor should have morphological surface complementary for the so called "pressor amines" ([7]). Activity of "pressor amines" on the smooth muscle fibers of blood vessels should depend on the degree of complementarity with the cellular receptor. Certain "pressor amines" of great complementarity with the receptor ([7]) are less active because their structure would occupy also hypotensive cellular receptors. Therefore, activity would result of an algebraic summation of effects, i.e. hypertension plus hypotension. Also, if a molecule has determined tachyphylaxis it must block functional effects through the same receptor of another molecule with less complementarity, even if it does not cause tachyphylaxis "per se". Inversely, if a given drug has induced tachyphylaxis these effects must disappear by the activity of other less

stable molecules, but with higher complementarity. According to the theory supported by Winder, Anderson and Parke ([23]) "crossed tachyphylaxis" could be explained accepting that the responsible drugs occupy the same hypertensive cellular receptor. The first agent that induces tachyphylaxis occupies all the receptors and then the response to a second agent is blocked. Therefore, we think that it is better to use the term tachyphylaxis to indicate the auto-extinction of pharmacological phenomena caused by repeated injections of chemicals at short intervals. Thus, it is preferable not to use the term "crossed tachyphylaxis", for its inadequacy in expressing a blockade action.

It is interesting to notice that there is no agreement to define what is generally called *facility* or *difficulty* to produce tachyphylaxis. This could be better understood if doses that would determine quantitatively an equal response on the same effector would be employed always. Thus, the number of equal doses repeated to produce tachyphylaxis would compel this concept and comparisons would be easier. In this paper it was considered that an amine had determined tachyphylaxis after it stopped producing hypertension or hypotension was present (in the case of "pressor amines", the number of equipressor doses used would be an index and is indicated in the last column of table I). It may be seen that Amphetamine is the substance that determines tachyphylaxis with more facility (index 3.42) and Phenylethylamine the one less tachyphylaxating (index 45.3).

It is difficult to know about the behavior of 2-methylamine-1-cyclopentylpropane, because the morphology of its molecule is highly complementary with one hypotensive receptor, and then induces very early hypotensions ([7]). Something similar happens with Butanefrine, that has a great affinity for the hypertensive receptor.

The best hypothesis to explain tachyphylaxis —especially for "pressor amines"— is the one that postulates that a given substance must stay in the receptor without change ([23]). Four facts would be necessary to accept it:

1) It must be indifferent to occupy all the receptors with a single dose or with the dose divided and repeated at short intervals.

2) That the necessary dose to occupy all the receptors must determine maximal hypertension.

3) Because of the morphology of the cellular receptor, it must be possible to block it with certain "pressor amines". In that case, other "pressor amines" would not be hypertensive substances.

4) The morphology of the receptor and of the "pressor amines" must indicate the way in which tachyphylaxis is going to be present.

These four premises were proven to be true. The two first points have been studied with Amphetamine, because it is a drug that easily induces tachyphylaxis. 1) It is indifferent to occupy all the receptors with a single dose or with fractional doses. Statistical studies of such dose of Amphetamine give an average of 1.6 $\pm$ 0.14 mg in 32 animals. It is possible to occupy all the receptors with fractional doses which were

repeated after the effect of a previous injection was over, or when blood pressure was stabilized. Results are abridged in table II.

The obtained results clearly indicate that statistically, there is no significative difference between values indicated in table II and 1.6 ± 0.14 mg. Similar values are found in the literature ([24]). These results agree with those of other authors ([23]) and that is why they think that tachyphylaxis is an additional phenomenon. It would indicate an additional occupation of receptors by the chemical substance.

TABLE II

Relation between fractional and total doses necessary to induce tachyphylaxis to Amphetamine.

Single doses mg	Total dose mg	Number of animals
0.25	1.2 ± 0.10	5
0.50	1.9 ± 0.31	6
1.00	2.3 ± 0.33	6
1.50	1.8 ± 0.30	15
2.00	2.0	3

TABLE III

Relation between Amphetamine doses and the degree of hypertension.

Doses mg	Hypertension mm Hg	Number of animals
0.25	11 ± 0.86	5
0.50	20 ± 1.92	16
1.50	24 ± 1.66	20
2.00 to 2.50	31 ± 2.00	17
5.00	23	3

2) The dose that occupies all the receptors determines maximal hypertension (see table III). As it was expressed above, mean doses of 1.6 ± 0.14 mg of Amphetamine occupy all the cellular receptors.

3) According to the hypertensive vascular receptor's morphology, it is possible to block it with some "pressor amines" ([7]).

4) The morphology of the receptor and of the "pressor amine" indicates if tachyphylaxis is going to be present or not and which will be its characteristics. Tachyphylaxis not only depends on surface complementarity, even if it plays the most important rôle on specificity and degree of hypertension. There are other factors related to the time of occupation of the specific receptor by the molecule. The ability of a drug to induce tachyphylaxis closely depends on how long the molecule is going to stay in the receptor. Therefore, it is most interesting to analyze shortly some of those factors.

A) *Physico-chemical factors.* — Van der Waals' forces act with equal intensity in any part of the molecule. Other intermolecular forces (ionic and polar forces and hydrogen bounds) do have an important rôle in stabi-

lizing molecule-receptor anchorage. Thus, the electronic structures of an adsorbent and the adsorbed substance may be quite important to determine how long the molecule is going to be trapped by the weak forces of van der Waals. Tachyphylaxis also depends on the intimate equilibrium of forces within its amine structure and on the presence or absence of oxhydryls in the molecule ([18]).

B) *Enzymatic factors.* — a) Inactivation of the so called "sympathomimetic amines" by phenol oxydase depends on molecule's polarity, which is determined by the number and position of oxhydryls in the bencenic ring and lateral chain ([2]). b) On the other hand, the well known *in vitro* and *in vivo* inactivation of Adrenaline by the action of monoamine-oxydase, being transformed into dihydroxy-phenyl-hydroxy-acetaldehyde, a pharmacologically inactive substance. Amine oxidase desaminizes any amine if nitrogen substitutes an hydrogen of an alpha carbon. Structures of isopropylamine type are not desaminized by amine oxydase, on the contrary, they inhibit the enzyme ([1]).

Accepting that the drug transformation *in situ* is caused by the amine oxydase, it could also be accepted that the receptor structure is complementary with an "activated complex of desamination". The conclusions of Elliot in 1905, that "Adrenaline disappears in tissues that it excites" ([1]), emphasizes the above expressed point of view. Also it must be noticed that in specificity and in mechanisms of action, statistical probability is important and thus, drug concentration is essential in tachyphylaxis induction. Statistical probability of the same drug also has to intervene in the occupation of hypertensive and hypotensive receptors, directly proportional to the drug degree of complementarity and both receptors. If the diverse factors, that theoretically modify tachyphylaxis are valorized numerally, it is found that these values coincide with the experimental results. These values were calculated by the rate at which equipressor doses were repeated to abolish hypertensive effect. Because of this, the discussed factors are believed to play an important rôle in tachyphylaxis and are not random factors. Also, because of the same results it is possible to predict that catecholic drugs may induce tachyphylaxis if they are injected at doses equivalent to other "pressor amines".

Experimental results ([7]) indicate that Win 3046 occupies a specific receptor for "depressor amines" in the vascular territory. "Pressor amines" of catecholic structure may occupy at the same time both the hypertensive and hypotensive receptors ([7]) but with different statistical possibilities. Therefore, it was tried to understand whether Adrenaline or Arterenol, after complete block of the specific hypotensive receptor, was more hypertensive. To know this and to have a better understanding of the hypertensive receptor morphology, repeated injections of Win 3046 were given until a perfect state of tachyphylaxis was obtained. That is to say, a substance which produces a specific blockade of the hypotensive receptor was used only. Under this condition, 10 micrograms of Adrenaline and 10 micrograms of Arterenol were injected. These drugs, under normal conditions determine a different degree of hypertension (see table I) i.e. Arterenol is statistically significantly more hypertensive than

Adrenaline. After tachyphylaxis to the hypotensive action of Win 3046, this relation is inverted: Adrenaline is more hypertensive than Arterenol (fig. 9).

SUMMARY

The effects of a great number of "pressor" and "depressor amines" were studied on blood pressure. Dosage is indicated in table I.

These studies were done in adult, non selected cats, anaesthetized with sodium pentobarbital, atropinized and with both cervical vagus sectioned. Blood pressure recordings were calibrated with a mercury manometer connected to the right carotid artery. All the substances were dissolved in distilled water and injected in the femoral vein. Pressor amines determine similar hypertension at the employed doses. Results were as follows.

1) All the substances, with the exception of catecholic structure amines and Neo-Synephrine induce tachyphylaxis to hypertension more or less easily. Once tachyphylaxis was established, further injections determine hypotension. The degree of hypotension varies according to different substances. Paredrine never induces hypotension.

2) Hypotensive amines also induce tachyphylaxis to hypotension, and once the phenomenon was established, other doses may cause hypertension. Win 3046 does not produce hypertension; Isuprel slight hypertension and Butanefrine a marked hypertension.

3) It is possible to observe tachyphylaxis to the hypotensive action of some pressor amines. Also, it is possible to induce tachyphylaxis to the hypertensive action of depressor amines.

4) The minimal doses that induce tachyphylaxis to the hypertensive action of a "pressor amine" of bencenic ring (Amphetamine was specially studied under this point of view) are able to block hypertensive responses of drugs chemically related. Furthermore, they can block responses of "pressor amines" of cyclic ring, aliphatic chain, and amines of dimetoxyphenylic structure. They can partially block responses of amines of phenolic ring and do not modify hypertension of catecholic amines. If the employed doses were far above minimal doses, it is possible to block partially the hypertensive action of catecholic amines.

5) After tachyphylaxis to the hypertensive action of amines of cyclic ring is established, hypertensive responses to similar compounds, to amines of bencenic ring and of aliphatic chain are absent. Same phenomenon is observed when tachyphylaxis is induced with amines of aliphatic chain or with those having dimetoxyphenylic ring.

6) Amines of phenolic structure, that with great facility induce tachyphylaxis (Paredrine) do block hypertensive responses of chemically similar compounds (except Neo-Synephrine) amines of bencenic ring, cyclic ring and of aliphatic chain.

7) Repeated doses of Isuprel or Butanefrine, and after a certain degree of tachyphylaxis to the hypertensive effect has been induced, induce a blockade of the hypertensive responses of amines of bencenic and phenolic ring. Hypertensive responses of catecholic amines are partially abolished. This blocking action is easier to obtain with Butanefrine than with Isuprel.

8) After tachyphylaxis to the hypotensive action of Isuprel has been determined, Butanefrine induces hypertension and Win 3046 does not modify blood pressure.

9) When tachyphylaxis to hypertensive action of Isopropylamine was induced, Butanefrine did not modify blood pressure or caused a slight hypertension.

10) After the hypotensive cellular receptor has been blocked by repeated doses of Win 3046, Adrenaline is more hypertensive than Arterenol. Under normal conditions this effect is inverse. These facts were interpreted according to physico-chemical principles that command specificity of biological phenomena, according to Pauling's experiments on the formation of antigen-antibody reaction. It has been deduced that "pressor amines" induce hypertension because they would be linked by surface complementarity, with only one specific cellular receptor adequate for them. Hypotension produced by "hypotensive amines" and by "hypertensive amines" after tachyphylaxis has blocked their hypertensive action would be caused by a bound between amines and hypotensive cellular receptors. According to these concepts, tachyphylaxis would be induced because a given amine would be linked by surface complementarity to the cellular receptor and stays there without any alteration all the time tachyphylaxis is present.

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ESTUDIO OSCILOGRÁFICO DE LA ACTIVIDAD ELÉCTRICA DE LOS QUIMIORRECEPTORES DEL SENO CAROTÍDEO EN EL PERRO

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EL ESTUDIO de los potenciales de acción del nervio de Hering, durante la excitación adecuada de los quimiorreceptores del glomus carotídeo es, probablemente, el método que puede proporcionar los datos más directos acerca de la fisiología de estos receptores. Pero al aplicar este método se presentan serios obstáculos. Según afirman Bronk y Stella en un trabajo de 1935 (4), no les fué posible hacer un estudio satisfactorio de la actividad quimiorreceptora de la región senocarotídea, por ser muy difícil separar en el electrograma del nervio de Hering los componentes barorreceptor y quimiorreceptor, debido a que los métodos utilizados para provocar cambios químicos en la sangre (asfixia, CO_2, hipoxia, etc.) producen, a su vez, alteraciones en la presión arterial.

En 1940, Schmidt y Comroe (9), después de hacer una revisión cuidadosa de los datos publicados sobre estudios oscilográficos de la actividad eléctrica de los quimiorreceptores del seno carotídeo, llegaron a la siguiente conclusión: "There is, of course, no doubt that the sinus nerve carries impulses from chemoreceptors and it is probable that these were responsible for the electrical activities studied in these experiments, but there is no actual evidence in any of the published reports to prove that to be the case" (pág. 142). Más adelante se afirma que, a pesar de las favorables condiciones anatómicas que presenta el perro para la preparación del seno carotídeo, y por lo tanto para el registro de las corrientes de acción del nervio de Hering, habían fracasado todos los intentos hechos para obtener en estos animales registros semejantes a los obtenidos en gatos y en conejos. Euler y Zotterman (5), dos años más tarde, publicaron un trabajo hecho en perros, en el cual se ilustran oscilogramas del nervio de Hering obtenidos durante la anoxia y las inyecciones de cianuro potásico, lobelina, etc.; resultados semejantes se comunican en los trabajos de Gernandt (7), publicados en el año 1946. Pero los oscilogramas de dichos trabajos, similares a otros publicados por estos mismos autores, presentan una actividad eléctrica de baja frecuencia y amplitud que, según afirmaciones de Schmidt y Comroe (9), parecen proceder del simpático, sin que nada demuestre que sean generados por los quimiorreceptores del seno carotídeo.

Así, en la bibliografía a nuestro alcance no se han encontrado datos que permitan dar una solución satisfactoria a las contradicciones existentes, por lo que se planteó este trabajo, en el cual se hace un nuevo estudio oscilográfico de la actividad eléctrica que se registra en el nervio de Hering del perro al excitar los quimiorreceptores de la región senocarotídea, inyectando en la carótida común soluciones de cianuro potásico.

MÉTODO

Se hicieron 40 experimentos en perros de 9 a 20 kg de peso, anestesiados con nembutal "Abbott" (35 mg por kg de peso, por vía intraperitoneal) o con uretano (1.25 g por kg de peso, en solución al 25 %) inyectando una tercera parte del volumen en la vena y las dos terceras partes restantes por vía intraperitoneal, o con cloralosa "Merck" (100 mg por kg de peso, en solución al 0.55 % en agua destilada) inyectada por vía endovenosa.

Para obtener una excitación adecuada de los quimiorreceptores del seno carotídeo se emplearon soluciones de cianuro potásico inyectadas directamente en la carótida común (Gessel y Owen [8], Winder [10, 11, 12]).

Con el fin de evitar la movilización de los barorreceptores, las inyecciones se hicieron a través de una aguja del número 20, administrando volúmenes comprendidos entre 1/10 y 7/10 de cm^3.

En el quimógrafo se registró la respiración, presión arterial y, algunas veces, presión endosinusal. Para el registro de respiración se empleó una cánula traqueal conectada a una cápsula inscriptora del tipo de Jackson; para el de la presión arterial, un manómetro de mercurio conectado a la arteria femoral; para la presión endosinusal, un manómetro de mercurio con cánula colocada en la arteria carótida externa.

El registro oscilográfico de la actividad eléctrica del nervio de Hering se hizo colocando este nervio, sin seccionar, sobre un par de electrodos de platino separados entre sí 5 mm. Se empleó un amplificador en *push-pull* de cuatro cascadas acopladas a resistencias y capacidades, diseñado por J. Beckwith. La salida de este amplificador se conectó a un oscilógrafo catódico Dumont, tipo 279, de dos rayos; para fotografiarlos se empleó la cámara Firechild tipo 314.

En los primeros experimentos se alcanzó la región senocarotídea a través de la línea que separa el borde externo del esternohioideo del borde interno del esternomastoideo; este último músculo, lo mismo que el digástrico, se secciona, haciéndose luego la resección del ganglio linfático, que cubre la superficie externa de la bifurcación carotídea. En estos preparados resultó difícil el registro de las corrientes de acción del nervio de Hering, por presentarse a menudo interferencias procedentes de las masas musculares cercanas. En la actividad eléctrica de muchos de estos músculos se observa ritmo respiratorio; otros, como consecuencia del trauma quirúrgico, fibrilan, dando una interferencia constante; pero todos suelen aumentar su actividad y, por lo tanto, la interferencia, al intensificarse la respiración como respuesta a la inyec-

ción de cianuro. Estas interferencias musculares son más intensas y frecuentes cuando la anestesia es ligera.

En tales condiciones se registran en el neurograma del nervio de Hering las interferencias musculares, junto con las señales procedentes de los receptores del seno carotídeo. Para separar estos dos componentes se interrumpió la transmisión en el nervio, pinzándolo fuertemente en ambos lados del lugar de derivación. La inyección de cianuro en estas condiciones produce el efecto que puede verse en los oscilogramas de la figura 1.

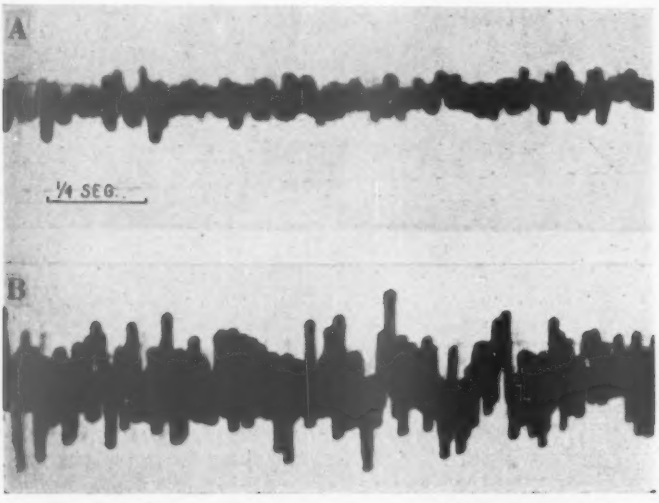


FIG. 1. — Perro; nembutal. Electrograma del nervio de Hering. Inyección en la carótida común de 350 microgramos de cianuro potásico, disueltos en 4/10 de cm^3. Efecto que produce sobre las interferencias musculares derivadas del nervio de Hering muerto. A. Antes de la inyección. B. Después de la inyección.

Como en estos preparados fracasaron todos los intentos para eliminar las interferencias musculares conservando sólo la actividad del nervio del seno; en el resto de los experimentos se optó por seguir otro procedimiento quirúrgico que permite alejar considerablemente del nervio de Hering a los músculos causantes de la interferencia.

Se hizo la incisión por la línea media, se seccionó y canuló la tráquea, seccionándose después el esófago y levantando al máximo, en dirección craneal, la laringe y la faringe. Se disecó el tejido conjuntivo que une la cara externa de la bifurcación carotídea con el nervio hipogloso y tejidos adyacentes; se disecó el conjuntivo que une la carótida interna a los ganglios nudoso y simpático cervical superior. Para eliminar las interferencias musculares, como precaución complementaria, se seccionaron en ambos lados las ramas musculares del nervio glosofaríngeo. Siempre fué preferible mantener la anestesia con profundidad suficiente.

RESULTADOS

Observando las precauciones que se indican en el método, no se aprecia ninguna actividad eléctrica en el nervio de Hering muerto. En el nervio de Hering normal se registra la actividad que aparece en el oscilograma A de la figura 2; y la inyección de 120 microgramos de cianuro, por kg de peso, disueltos en tres décimas de cm^3, produce el efecto que se observa en el oscilograma B. Las señales de los oscilogramas que se

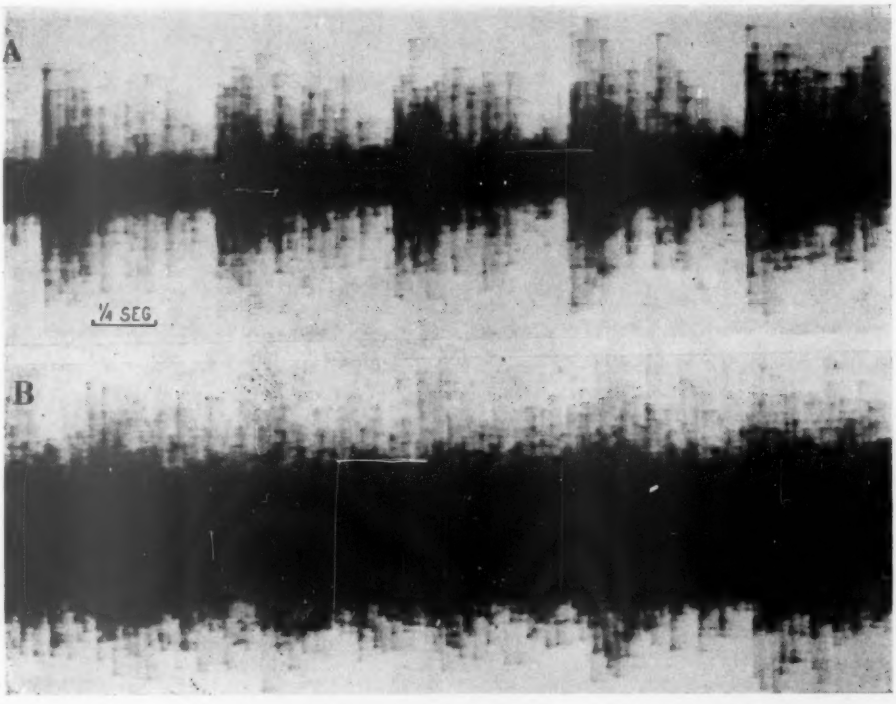


FIG. 2. — Perro; nembutal. Electrograma del nervio de Hering. Inyección de 120 microgramos por kg de peso, de cianuro potásico, disuelto en 3/10 de cm^3 de suero fisiológico; efectos que produce sobre las corrientes de acción del nervio de Hering. A. Antes de la inyección. B. Después de la inyección.

ilustran en esta figura son, sin lugar a dudas, impulsos nerviosos transmitidos por el nervio de Hering, pero es preciso aclarar cuáles de estas señales se originan en los barorreceptores y cuáles en los quimiorreceptores de la región senocarotídea.

La actividad barorreceptora se presenta en forma de agrupaciones triangulares con ritmo cardíaco que desaparecen al pinzar la carótida común. Cuando se cambia la presión en la región del seno carotídeo, la

configuración de estas agrupaciones cambia inmediatamente en forma semejante a como lo hacen las señales del nervio aórtico (Alvarez-Buylla, [2]).

La inyección de cianuro motiva la aparición de gran número de nuevas señales, independientes de las agrupaciones barorreceptoras y que podrían considerarse originadas por los quimiorreceptores.

Para asegurarse que estas señales no son debidas al aumento de la actividad barorreceptora como consecuencia de la inyección, se controló

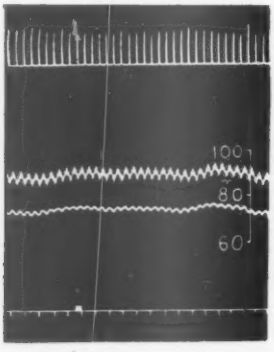


FIG. 3. — Perro; cloralosa. Registro quimográfico de respiración, presión arterial y presión endosinusal. En la señal, inyección de 5 cm³ de suero fisiológico; obsérvese que no se produce ninguna variación en la presión endosinusal.

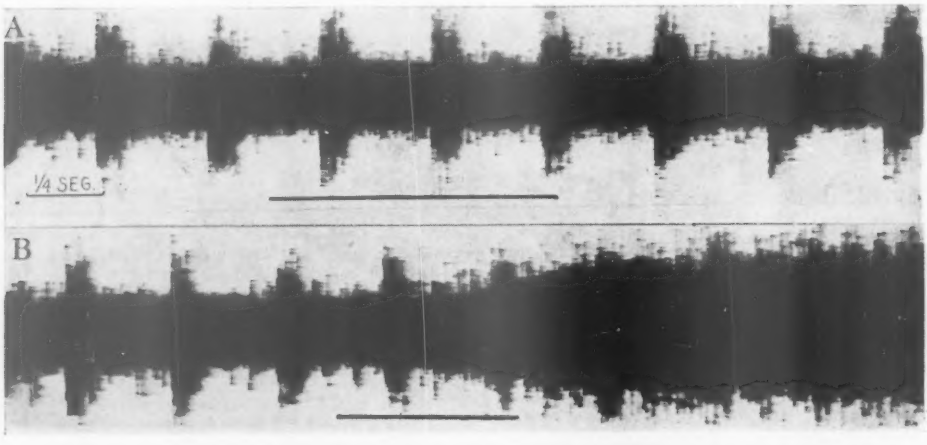


FIG. 4. — Perro; cloralosa. Electrograma del nervio de Hering. En *A*, inyección de 5 cm³ de suero fisiológico; en *B*, inyección de 240 microgramos por kg de peso de cianuro potásico disuelto en 6/10 de cm³ de suero fisiológico.

la presión en el seno carotídeo, canulando la carótida externa. En estas condiciones, 5 cm^3 de suero fisiológico inyectados en la carótida común, con la mayor rapidez que permite una aguja del número 20, no produce ningún cambio en la presión endosinusal (figura 3) y ninguna variación de la actividad barorreceptora (A de la figura 4). Sin embargo, la inyección de 240 microgramos de cianuro por kg de peso, disueltos en 0.6 de cm^3 (B de la figura 4) produjo el efecto que se ve en el oscilograma. Este efecto se prolonga 30 segundos. Sin embargo, cuando las señales proceden de los barorreceptores, persisten sólo mientras dura el cambio de presión. Estos hechos hacen suponer que las señales provocadas por las inyecciones de cianuro son procedentes de los quimiorreceptores.

Dividiendo longitudinalmente el nervio de Hering con objeto de poder controlar la actividad eléctrica de un número reducido de fibras, se pudo observar que en los distintos grupos había, casi siempre, actividad de carácter barorreceptor y de carácter quimiorreceptor. Sólo en algunas ocasiones y en forma casual, se consiguieron grupos de fibras en los que no se observó ningún indicio de actividad barorreceptora y, sin embargo, la inyección de cianuro provocó la aparición brusca de señales; como ejemplo se ilustran los oscilogramas que aparecen en la figura 5.

Este hecho demuestra la existencia de receptores sensibles a los excitantes químicos e insensibles a los cambios de presión. Como ya Bronk y Stella [4] demostraron la existencia de otros receptores sensibles a las variaciones de presión y prácticamente insensibles a los cambios químicos, hay que admitir la existencia en la región senocarotídea de dos clases de receptores independientes: barorreceptores y quimiorreceptores.

Si las reacciones respiratorias provocadas por el cianuro son las respuestas del sistema nervioso central a las señales registradas en el nervio de Hering, entre estas señales y las respuestas respiratorias debe existir una determinada relación temporal que justifique esta suposición de causa y efecto.

Con objeto de estudiar estas relaciones temporales se recurrió al registro de la respiración y presión arterial en el quimógrafo, simultáneamente con el registro oscilográfico de la actividad del nervio del seno carotídeo. Para obtener una sincronización perfecta entre estos dos registros, se conectaron en paralelo las señales electromagnéticas que marcan el tiempo y la excitación en el quimógrafo, con la lámpara de neón que hace las marcas correspondientes en el registro oscilográfico (fig. 6). Comparando el registro quimográfico con los oscilográficos vemos que las señales aparecen en el nervio casi inmediatamente después de la inyección de cianuro (oscilograma *a)* alcanzan su máxima a los ocho segundos (oscilograma *b)* y no desaparecen hasta 42 segundos después de haber inyectado (oscilograma *c)*. La hiperpnea que se registra en el quimograma de esta misma figura se inicia 7 segundos después de la inyección, y desde este momento sigue un curso paralelo al de las señales registradas en los oscilogramas, observándose en cualquier momento una correspondencia satisfactoria entre la cantidad de señales y la magnitud del efecto.

Tales hechos confirman que estas señales tienen su origen en los quimiorreceptores de la región senocarotídea y constituyen el componente aferente del reflejo respiratorio que provoca el cianuro.

Existe una determinada relación entre la cantidad de cianuro que se inyecta y la magnitud del fenómeno eléctrico que se registra en el nervio; como también ocurre igual entre las dosis de cianuro y la magnitud de la respuesta respiratoria, nos pareció interesante aprovechar este procedimiento de registros simultáneos en el quimógrafo y el oscilógrafo para estudiar paralelamente las relaciones entre dosis de cianuro, fenómeno eléctrico registrado en el nervio y magnitud de las respuestas obte-

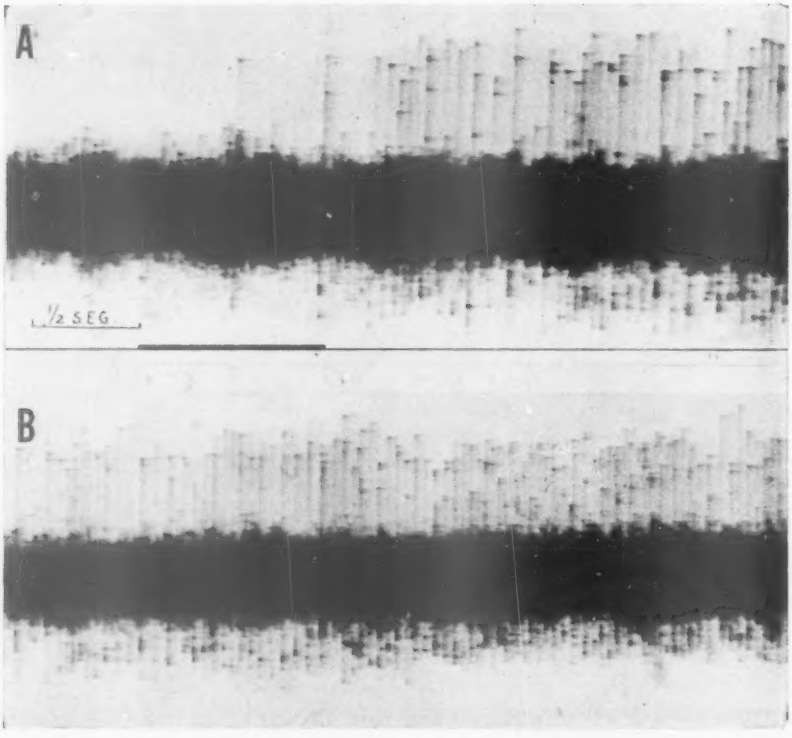
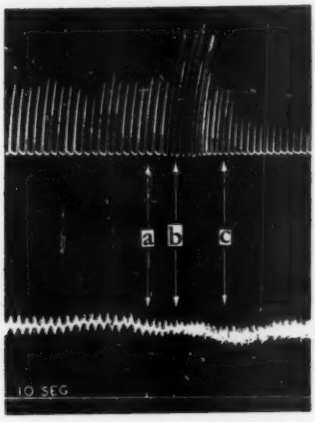


FIG. 5. — Perro; nembutal. Electrograma de un número reducido de fibras del nervio de Hering, en las que no se aprecia actividad de origen barorreceptor. En *A*, inyección de 120 microgramos por kg de peso, de cianuro potásico disuelto en 3/10 de cm^3 de suero fisiológico. *B*, continuación de *A*.

nidas en el quimógrafo. Así, se hicieron la serie de oscilogramas y quimogramas que se ilustran en las figuras 7 y 8, en donde se pudo confirmar el paralelismo existente entre la intensidad de las respuestas respi-



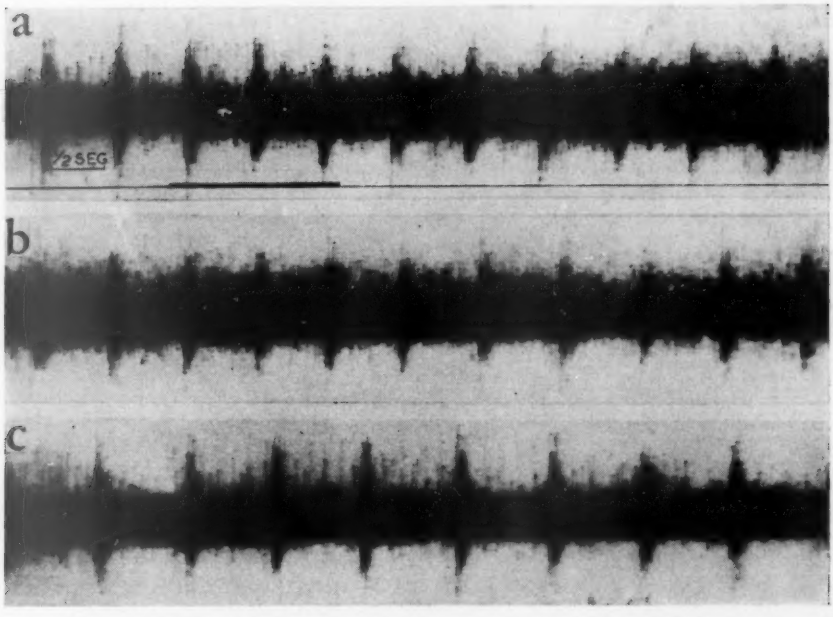


FIG. 6. — Perro; uretano. *Arriba:* Registro quimográfico de respiración y presión arterial. En *a*, inyección de 124 microgramos por kg de peso de cianuro potásico, disuelto en 2/8 de cm^3 de suero fisiológico; *b*, 8 segundos después de *a; c*, 22 segundos después de *b*. *Abajo:* Electrogramas del nervio de Hering, que corresponden a los puntos marcados con *a*, *b*, y *c* del quimograma. Las señales aparecen en *a*, alcanzan su intensidad máxima en *b* y desaparecen en *c*.

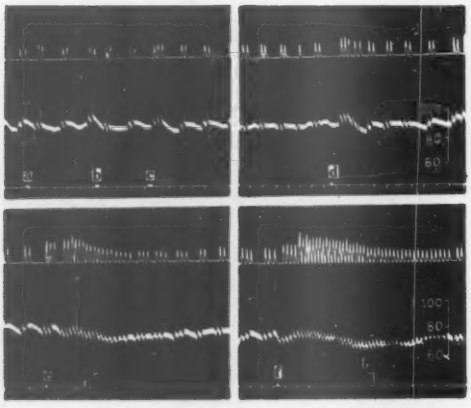


FIG. 7. — Perro; nembutal. Registro quimográfico de respiración y presión arterial. Efectos que sobre la presión arterial y respiración producen las distintas dosis de cianuro potásico inyectadas en la carótida común.
En *a:* 20.4 microgramos por kg de peso, disueltos en 2/10 de cm^3 de suero fisiológico. En *b*: 30.6 microgramos por kg de peso, disueltos en 3/10 de cm^3 de suero fisiológico. En *c:* 40.8 microgramos por kg de peso, disueltos en 4/10 de cm^3 de suero fisiológico. En *d:* 91.8 microgramos por kg de peso, disueltos en 6/10 de cm^3 de suero fisiológico. En *e:* 183 microgramos por kg de peso, disueltos en 5/10 de cm^3 de suero fisiológico. En *f:* 285 microgramos por kg de peso, disueltos en 4/10 de cm^3 de suero fisiológico.

ratorias y la de las señales producidas por la inyección de cianuro a que antes hicimos referencia. Dicho paralelismo se rompe cuando la cantidad de cianuro es superior a 150-180 microgramos por kg de peso, lo que parece deberse a que el cianuro, en tales cantidades, llega a las otras zonas quimiosensibles con una concentración supraumbral.

En los registros se utilizó el nervio íntegro, pues, como ya hizo notar Adrián, la respuesta refleja no puede depender de un pequeño número de receptores sino de la integral de toda la zona receptora. Como en los electrogramas del nervio íntegro es imposible contar la frecuencia, teniendo en cuenta que se trata de estudiar la actividad de receptores se pensó que el criterio fisiológico más apropiado para obtener datos cuantitativos, que facilitaran la comparación, era medir el tiempo durante el cual se mantienen las descargas de los quimiorreceptores al ser excitados con distintas dosis de cianuro potásico. De esta manera se construyeron las curvas que aparecen en la figura 9. El efecto suele comenzar cuando la cantidad de cianuro es superior a 5 ó 6 microgramos por kg de peso, pero es tan pequeño que hace difícil su medida; al ir aumentando la dosis de cianuro el efecto aumenta rápidamente, hasta alcanzar el máximo cuando la dosis es aproximadamente 150 microgramos por kg de peso, nivel al que se mantiene hasta sobrepasar los 300 microgramos por kg de peso; desde este límite, la generación de impulsos se hace cada vez más corta y la curva cae rápidamente; si la dosis es

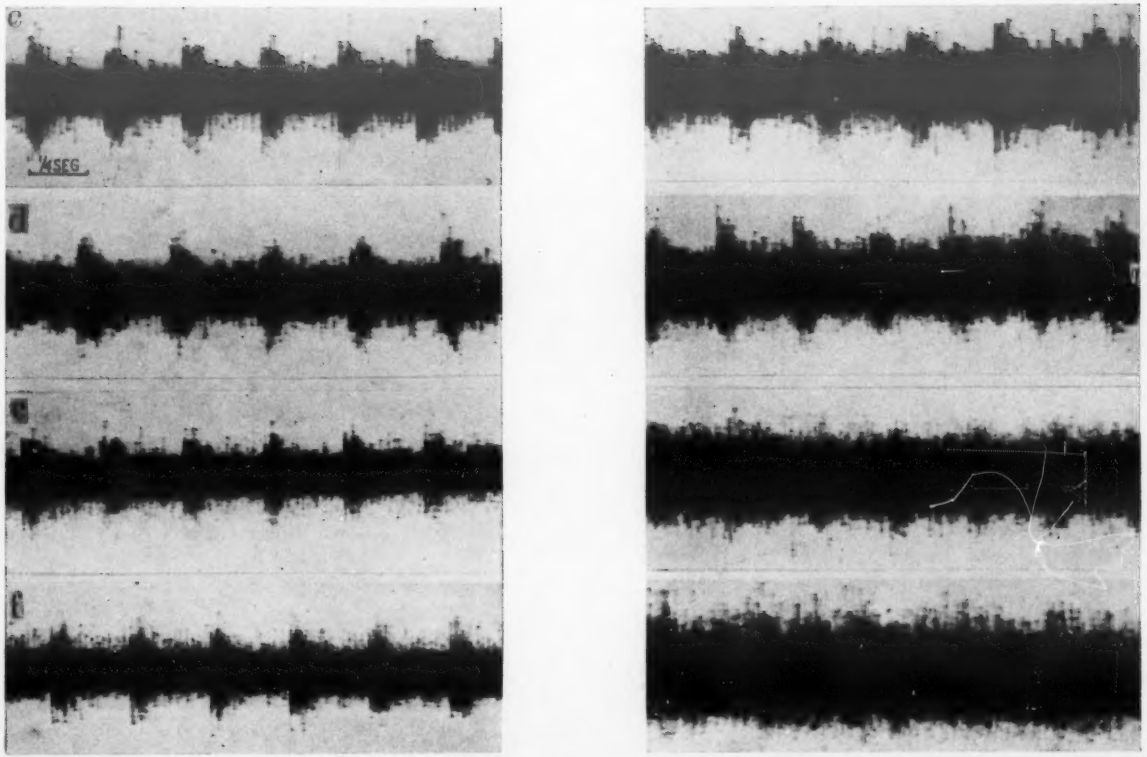


FIG. 8. — El mismo experimento de la figura 7. Registros: Electrogramas del nervio de Hering correspondientes a los puntos marcados con *c*, *d*, *e* y *f* en los quimogramas de la figura 7. Los oscilogramas de la izquierda fueron hechos antes de la inyección, los de la derecha después de la inyección. Obsérvese la relación entre la magnitud de las respuestas respiratorias registradas en los quimogramas de la figura 7 y la cantidad de señales que se observan en los oscilogramas correspondientes.

superior a 450 ó 500 microgramos por kg de peso, desaparece toda evidencia de actividad quimiorreceptora.

Como se observó cierta diferencia en la actividad eléctrica del nervio de Hering al inyectar cianuro, según la substancia anestésica empleada en el animal, se repitieron los últimos experimentos utilizando cloralosa en vez de uretano. Aplicando los mismos métodos y criterios se obtuvieron los resultados que se sintetizan en la curva B de la figura 9.

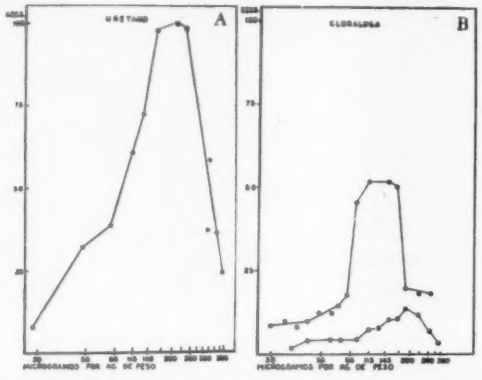


FIG. 9. — Curvas que expresan la relación entre las dosis de cianuro potásico inyectadas en la carótida común y la duración de la actividad quimiorreceptora que provocan. En *A*, perros anestesiados con uretano. En *B*, perros anestesiados con cloralosa. La curva construída con puntos negros es la correspondiente a una anestesia ligera; la construída con puntos blancos a una anestesia profunda.

De la comparación de la curva A, obtenida en animales anestesiados con uretano, con la curva B, obtenida en animales anestesiados con cloralosa, se deduce que la actividad de los quimiorreceptores es más intensa con el uretano que con la cloralosa. Por otra parte, en los perros anestesiados con cloralosa se puede observar una mayor actividad quimiorreceptora cuando la anestesia es ligera. En B de la figura 9, la curva trazada con puntos negros corresponde a un nivel ligero de anestesia; la curva trazada con puntos blancos, a una anestesia profunda.

DISCUSIÓN

Los principios anatómicos y fisiológicos más sólidamente establecidos en la actualidad hacen suponer que la información procedente de los receptores localizados en la región senocarotídea se transmite por la rama del nervio glosofaríngeo, conocida con el nombre de nervio de Hering o nervio del seno, y que los somas celulares de las fibras que lo integran son células bipolares que se encuentran en el ganglio petroso del nervio glosofaríngeo. Siendo así, las señales aferentes que transmite deben corresponder en su mayoría a las fibras A y B de dichas neuronas y presentar el aspecto clásico de descargas nerviosas, cualquiera que sea la complicación del aparato receptor.

Sin embargo, los registros que presentan Euler y Zotterman ([5]) y Gernandt ([7]) podrían interpretarse como actividad procedente de fibras nerviosas post-ganglionares, obligando a suponer que el cuerpo celular de estas fibras estuviera localizado en el mismo glomus, hecho que parece descartado por los experimentos del mismo Euler, quien comprobó que después de la sección del nervio del seno degeneran las fibras que quedan en comunicación con el glomus ([5]). En los oscilogramas que se presentan en este trabajo puede verse que, al excitar los quimiorreceptores por la inyección de cianuro en la carótida común, se registran señales nerviosas en el nervio de Hering, que parecen proceder en su mayoría de fibras A y B. Como, por otra parte, en nuestros experimentos quedaron eliminadas las posibilidades de interferencia de potenciales ajenos al nervio y también las variaciones de presión en el seno, es lógico suponer que la actividad eléctrica registrada procede de los receptores quimiosensibles de la bifurcación carotídea.

En otro trabajo, Alvarez-Buylla ([3]) observó que en los perros anestesiados con uretano se produce taquipnea, y bradipnea, cuando se utiliza cloralosa; este hecho podría ser consecuencia de la mayor actividad quimiorreceptora que hemos observado en los perros anestesiados con uretano.

Ajustándose a los conceptos de la escuela de Anogin *(Centro y Periferia,* 1935,[1]) y a lo expuesto por Gellhorn en su monografía *(Autonomic Regulation,* 1942,[6]) se podría explicar que la mayor actividad de los quimiorreceptores observada con el uretano, fuera la causa de una mayor actividad respiratoria que produciría una disminución del tono vasomotor. Por el contrario, en los perros anestesiados con cloralosa, al disminuir la acción tónica de los quimiorreceptores, sobreviene la consiguiente depresión respiratoria, que sería compensada por una exaltación del tono vasomotor; estas ideas se ven confirmadas, en parte, por el hecho de convertirse la hiperpnea causada por el uretano en depresión respiratoria cuando se eliminan las zonas reflexogénicas; sin embargo, en los perros anestesiados con cloralosa la eliminación de estas zonas no produce ningún cambio notorio sobre la frecuencia respiratoria (Alvarez-Buylla, [3]).

RESUMEN

1. Se han registrado las señales que surgen en el electrograma del nervio de Hering al inyectarse cianuro en la carótida común.

2. Los controles realizados permiten afirmar que estas señales proceden de los quimiorreceptores senocarotídeos.

3. Se determinó la relación existente entre la actividad de los quimiorreceptores de la bifurcación carotídea y las dosis de cianuro inyectadas en la carótida común.

4. Se han estudiado las relaciones existentes entre estas señales y las reacciones respiratorias que provocan.

5. Se ha comparado la actividad de los quimiorreceptores senocarotídeos de los perros anestesiados con cloralosa, con la de los perros anestesiados con uretano.

6. Los datos obtenidos de esta comparación han permitido encontrar nuevas bases para explicar la distinta forma de acción de la cloralosa y el uretano sobre la respiración y el tono vasomotor.

SUMMARY

The action potentials of Hering's nerve were recorded in 40 experiments on dogs anesthetized with nembutal, chloralose or urethane, after injections of K cyanide into the common carotid artery. These injections elicit abundant discharges that subside in 15 to 60 s, depending on the dose of cyanide administered (fig. 2).

It was possible to isolate groups of fibers arising from receptors insensitive to pressure changes but highly sensitive to cyanide (fig. 5).

Simultaneous records of the action potentials of Hering's nerve, respiratory movements and blood pressure allowed the correlation of the dose of cyanide injected with the degree of nerve discharge and the respiratory responses (figs. 7 and 8).

It was found that the activity of the chemoreceptors is greater in dogs anesthetized with urethane than in those anesthetized with chloralose (fig. 9).

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EFECTO DE LA DENERVACIÓN SOBRE LA EXITABILIDAD DE LA MEMBRANA NICTITANTE FRENTE A LA ACCIÓN DE ALGUNOS FÁRMACOS

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NUMEROSAS publicaciones han evidenciado que un efector cualquiera, denervado, aumenta su sensibilidad frente a excitantes químicos. Este hecho general, conocido bajo la denominación de "ley de denervación", ha sido definido muy recientemente por Cannon y Rosenblueth de la siguiente manera: "Cuando en una serie funcional de neuronas se secciona uno de sus elementos, la denervación total o parcial que se sigue de los elementos consecuentes de la cadena causa una hipersensibilidad de todos los elementos distales, incluyendo aquéllos no denervados, y de los efectores, cuando los hay, a la acción excitatoria o inhibitoria de agentes químicos o impulsos nerviosos. La sensibilización es mayor para los elementos que siguen inmediatamente al neurón seccionado y disminuye progresivamente hacia los elementos más distales" (5).

A pesar de que la ley de denervación es ampliamente aceptada, existen numerosas excepciones, como las descritas por Rosenblueth (13) en la membrana nictitante (m.n.) para la ergotamina y para la acción potenciadora de la cocaína sobre la adrenalina, siendo los efectos de estas drogas mayores en la m.n. de menor tiempo de denervación. Otras excepciones han sido descritas por Altamirano y Huidobro (2) para el cloruro de potasio y prostigmina en el músculo esquelético; por Altamirano, Fernández y Luco (1) para la ergotamina y dihidroergotamina, que se mostraron más activas en la m.n. de menor tiempo de denervación. Igual fenómeno observaron estos autores para la acción depresora de las drogas curarizantes y para la acción potenciadora de la prostigmina. Lockett (9) encuentra que la sensibilidad de la m.n. desarrollada frente a algunas aminas simpaticomiméticas es diferente si la denervación es pre o postganglionar; en este último caso la denervación disminuye la sensibilidad a la anfetamina.

Huidobro y Poblete (7) observaron que la sensibilidad del cuerno uterino denervado frente al extracto post hipofisario, ergotamina, histamina y cloruro de bario era menor que la del cuerno recientemente denervado; igual cosa observaron en el músculo esquelético empleando prostigmina, veratrina y cafeína. Pudieron comprobar, en este último efector, que la excepción al proceso de sensibilización por denervación era aparente, ya que sólo se presentaba cuando existen, aunque seccionadas, las fibras nerviosas susceptibles de ser excitadas por las drogas.

Se ha estimado de interés estudiar la acción de algunos de los fármacos empleados por estos últimos autores sobre un efector autonómico y comprobar si se reproducía el fenómeno descrito en el párrafo anterior para el músculo esquelético. Para ello se eligió la fibra muscular lisa de la m.n. inervada por fibras simpáticas adrenérgicas, empleándose drogas generalmente aceptadas como excitantes directas de la fibra muscular.

MÉTODOS

Se emplearon gatos que pesaban 2 y 3 kg. Se anestesiaron con 1 cm^3 por kg de peso, intra peritoneal, de una solución de uretano al 25 % que contenía 3.3 % de pentobarbital sódico (Nembutal, "Abbott").

Se efectuaron dos series experimentales. En ambas se denervó una m.n. por extracción aséptica del ganglio cervical superior bajo anestesia etérea entre 4 y 73 días antes de los experimentos. La otra membrana fué, en la primera serie, denervada inmediatamente antes de la experiencia y, en la segunda serie, entre 72 y 120 horas antes.

Las contracciones de la m.n. se inscribieron isotónicamente en un quimógrafo ahumado según la siguiente técnica: se enuclearon ambos ojos, y se unieron los bordes libres de la m.n. mediante un hilo de seda a los brazos cortos de sendas palancas inscriptoras. Se puso especial cuidado para que la amplificación —que en general fué de 1:15— y la tensión de ambas palancas fueran iguales.

Las drogas se inyectaron por la vena femoral en un volumen nunca superior a 1 cm^3. Cuando se inyectaron varias drogas en un mismo animal se dejó pasar un tiempo prudencial (25 ó más minutos) para que el efecto de la droga anterior hubiera pasado; además, el orden de inyección de las diferentes substancias se alteró en los distintos animales.

Las drogas empleadas fueron: Prostigmina "Roche" en una solución acuosa al 1:2 000; cloruro de bario "Merck" al 1:100 disuelto en agua destilada; veratrina, en solución alcohólica al 5 % y extracto post-hipofisario, concentrado en ocitosina, Pituitrol "Sanitas" de 10 U por cm^3.

RESULTADOS

La sensibilidad de la m.n. se valoró de acuerdo con la amplitud de sus contracciones ante dosis ligeramente subumbrales, ya que, como es sabido, las contracciones máximas de una m.n. denervada pueden ser iguales a las de su homóloga contralateral normal.

Ya que la m.n. puede presentar contracciones espontáneas ([12]) se ha tenido especial cuidado en considerar sólo aquellos experimentos en que las contracciones eran evidentemente relacionadas con la inyección de la droga; los casos dudosos se desecharon.

Los resultados se han agrupado en dos series, de acuerdo con las condiciones experimentales.

I. — *Efecto de las drogas sobre la m.n. crónicamente denervada (4 a 73 días) en comparación con su homóloga contralateral denervada inmediatamente antes del experimento.*

A) *Extracto post-hipofisario.* Se usó en dosis que fluctuaron entre 1 y 10 U; la dosis media fué de 3.75 U. Se han observado 3 tipos de respuestas.

a) Contracción pura: se ha presentado 1 vez en 28 inyecciones de extracto hipofisario y correspondió a una membrana denervada inmediatamente antes de la experiencia.

b) Contracción precedida de una pequeña relajación: la depresión es escasa y de muy corta duración y va seguida de una contracción o de un aumento de *tonus* de la membrana (fig. 1). Este tipo de respuesta ha sido la más frecuente: se ha observado en 21 de 28 inyecciones; 13

Fig. 1

Fig. 2

FIG. 1. — Menor efecto del extracto post hipofisario sobre la membrana nictitante crónicamente denervada.

1ª línea (de arriba a abajo, en ésta como en las demás figuras): membrana nictitante testigo. 2ª línea: membrana nictitante crónicamente denervada (10 días). 3ª línea, en la señal: 1 U de extracto hipofisario en la vena femoral. 4ª línea: tiempo en minutos (en ésta como en las demás figuras la desigualdad en la longitud de las señales corresponde a cambios de velocidad del quimógrafo).

FIG. 2. — Menor efecto de la prostigmina sobre la membrana nictitante crónicamente denervada.

1ª línea: membrana testigo. 2ª línea: membrana nictitante crónicamente denervada (14 días). 3ª línea: inyección de 250 microgramos de prostigmina en la vena femoral. 4ª línea: tiempo en minutos.

pertenecen a m.n. recientemente denervadas y 8 a membranas crónicamente denervadas.

c) Relajación pura: se ha observado en 6 de las 28 inyecciones; siempre correspondió a membranas crónicamente denervadas.

Para apreciar la sensibilidad de la m.n. al extracto post-hipofisario se consideró exclusivamente la intensidad de la contracción o del aumento de tonus sin tomar en cuenta la relajación previa. La respuesta se catalogó como negativa cuando sólo hubo relajación.

En 8 de los 10 animales estudiados hubo un acortamiento muscular de mayor amplitud de la m.n. testigo (fig. 1). En una ocasión la respuesta de la m.n. crónicamente denervada fué mayor y en otra las respuestas de ambas fueron iguales.

Es importante hacer notar que el músculo de la m.n. es poco sensible al extracto post-hipofisario en comparación con otras estructuras musculares lisas: útero, intestino, vasos sanguíneos, etc., ya que, a veces,

Fig.3

Fig.4

FIG. 3. — Menor efecto del cloruro de bario sobre la membrana nictitante crónicamente denervada.

1ª línea: membrana testigo. 2ª línea: membrana crónicamente denervada (24 días). 3ª línea: inyección de 10 miligramos de cloruro de bario en la vena femoral. 4ª línea: tiempo en minutos.

FIG. 4. — Menor efecto de la veratrina sobre la membrana nictitante crónicamente denervada.

1ª línea: membrana testigo. 2ª línea: membrana crónicamente denervada (7 días). 3ª línea: inyección de 0.25 miligramos en la vena femoral. 4ª línea: tiempo en minutos.

aún con 10 U sólo se obtuvieron contracciones de amplitud muy escasa.

B) *Prostigmina.* — Las dosis fluctuaron entre 30 y 300 microgramos, siendo la de 50 microgramos la más empleada.

Sobre un total de 20 inyecciones, en 17 casos se obtuvo una contracción y en 3 un aumento de *tonus*. Esto último se manifestó en m.n. crónicamente denervadas, lo que se puede apreciar en la figura 2.

La prostigmina produjo mayor contracción en la m.n. testigo (fig 2) en 8 de los 10 animales empleados. En un caso la contracción fué de mayor amplitud para la m.n. crónicamente denervada, y en otro las respuestas fueron iguales.

Estos resultados podrían considerarse como discordantes con los de Rosenblueth ([13]) quien encuentra más sensible la m.n. crónicamente denervada frente a la fisostigmina, droga de efectos muy similares a la prostigmina ([6]).

B) *Cloruro de bario.* — Se usó en dosis media de 5 miligramos, siendo la variación de las dosis entre 2 y 10 miligramos.

La contracción fué mayor (fig. 3), o sólo existió en la m.n. denervada inmediatamente antes del experimento en 8 y 10 animales. En los 2 experimentos restantes la membrana crónicamente denervada respondió con una contracción mayor.

D) *Veratrina* —.La dosis usada varió entre 0.25 y 1 miligramo, empleándose con mayor frecuencia la cantidad de 0.5 miligramos.

La respuesta de la m.n. se caracteriza por una contracción rápida, seguida de una relajación de mayor duración.

Sobre 10 animales se tuvo mayor respuesta en la m.n. testigo en 7 casos (fig. 4). Demostró mayor sensibilidad la m.n. crónicamente denervada sólo en 1 caso e igualó a la m.n. testigo en otro.

En resumen, estas cuatro drogas producen en igualdad de dosis una menor contracción de la membrana denervada entre 4 y 73 días antes de la experiencia que aquella cuyo ganglio cervical superior se extirpó inmediatamente antes de la misma. Como se ha indicado, existen algunas excepciones, no habiendo relación entre el tiempo de denervación y éstas.

II. — *Efecto de las drogas sobre la m.n. crónicamente denervada (7 a 34 días) en comparación con su homóloga contralateral denervada entre 72 y 110 horas antes de la experiencia.*

Para eliminar la posibilidad de que las drogas actuaran sobre las fibras nerviosas post ganglionares no degeneradas de la membrana testigo, y se enmascarara la verdadera sensibilidad de las fibras musculares lisas, se dejó trancurrir, entre la extracción del ganglio de esta m.n. y el experimento, un tiempo corto (72 a 110 horas) pero suficiente como para permitir una degeneración nerviosa, la que se acompaña de pérdida de la excitabilidad nerviosa.

A) *Extracto post-hipofisario.* — La dosis efectiva media fué de 5 U, dosis algo superior que la media usada en la serie anterior. Respecto a la variedad de respuesta, cabe hacer notar lo siguiente:

a) No hubo contracción pura, hecho que en la serie anterior se observó en una oportunidad en una m.n. recientemente denervada.

b) Las contracciones precedidas de una pequeña relajación, a semejanza de la serie anterior, se presentaron con frecuencia similar en ambas membranas.

c) Las relajaciones puras, que se describieron en la primera serie como características de la m.n. crónicamente denervada, se repartieron sin preferencia entre ambas membranas.

La sensibilidad de la membrana fué mayor en el efector crónicamente denervado en 3 casos sobre 7. Hubo igualdad de contracción en 2 casos, y en otros 2 la respuesta mayor fué en la m.n. de menor tiempo de denervación.

B) *Prostigmina.* — Se empleó en la misma dosis media que en la serie anterior (50 microgramos). Los efectos fueron los siguientes: sólo en 3 casos sobre 8 se vió el fenómeno predominante de la primera serie, es decir, una mayor contracción en la membrana de menor tiempo de denervación. En los experimentos restantes se vió un franco predominio de la contracción de la m.n. crónicamente denervada en 3 casos, e igualdad en 2 casos.

C) *Cloruro de bario.* — La dosis media empleada fué de 4.2 miligramos. Al revés de lo notado en la primera serie, la m.n. testigo mostró mayor sensibilidad en la minoría de los casos: 3 sobre 7. La membrana crónicamente denervada sobrepasó en sensibilidad a la anterior en 2 casos y la igualó en otros 2.

D) *Veratrina.* — Los resultados con una dosis media de 0.46 miligramos fueron: sobre 8 animales, 3 exhibieron mayor contracción de la m.n. denervada 72 a 110 horas antes. La sensibilidad de la m.n. crónicamente denervada fué mayor en 3 casos, e igual en 2.

En resumen, en esta segunda serie se ve desaparecer el franco predominio de la sensibiildad de la m.n. testigo que se observó en la primera serie, presentándose, ahora, con mayor frecuencia, los casos en que la sensibilidad de ambas membranas es igual.

Discusión

Dado que los resultados han sido similares tanto para drogas vasopresoras como para las vasodepresoras, se puede descartar la intervención de cambios de la presión arterial en estos fenómenos.

Los resultados de la primera serie experimental indican que la denervación de la fibra muscular lisa de la m.n. del gato, hecha entre 4 y 73 días antes de la experiencia, no exterioriza, con dosis ligeramente superiores a la umbral de extracto post hipofisario, prostigmina, cloruro de bario y veratrina, un estado de hipersensibilidad tal como podría esperarse. Por el contrario, la contracción mayor correspondió, en la mayoría de los casos, a la membrana testigo, denervada inmediatamente antes de la experiencia (ver cuadro 1).

Se ha observado un hecho similar en el músculo esquelético cuando se usa veratrina, prostigmina y cafeína ([7]). El fenómeno se explicó suponiendo que en el músculo testigo —denervado inmediatamente antes de la experiencia— la suma de las excitaciones producidas por la droga sobre la fibra muscular estriada y sobre el nervio motor primaría sobre la

sensibilización del músculo crónicamente denervado, cuyo nervio ha degenerado.

La estimulación de un nervio motor por la acción de la prostigmina ha sido demostrada en forma directa por Masland y Wington (11) y por Arvanitaki y Fessard (4) para la veratrina. No ha sido descrita esta acción ni para el extracto post-hipofisario ni para el cloruro de bario; tampoco se ha descrito que la prostigmina o la veratrina puedan esti-

CUADRO I

Efecto de las drogas sobre la membrana nictitante crónicamente denervada en comparación con la testigo denervada inmediatamente antes del experimento.

Denervación en días	Extr. post-hipofisario		Prostigmina		Veratrina		Cl_2Ba	
	T.	C.	T.	C.	T.	C.	T.	C.
4	+		+		+		+	
4		+	+		+		+	
10	+		+		+		+	
14	+		+			=	+	
14	+			=		+		+
15	+		+		+		+	
24		=	+		+		+	
31	+			+		+		+
35	+		+		+		+	
73	+		+		+		+	

T.: Membrana nictitante testigo (denervada inmediatamente antes de la experiencia).
C.: Membrana nictitante crónicamente denervada (los días de denervación están indicados en la primera columna del cuadro).
+. Membrana nictitante que se contrae más intensamente.
=. Igual contracción en ambas membranas nictitantes.

mular los filetes nerviosos del sistema autonómico. No obstante, los resultados de la segunda serie permitirían sospechar que las excepciones a la ley de denervación observadas en la primera serie fuesen sólo aparentes, ya que se podrían explicar, como en el trabajo citado (7) por una posible acción excitadora de dichas drogas sobre las fibras nerviosas, en este caso las fibras post-ganglionares simpáticas.

Al examinar el cuadro 2 llama la atención que la inversión de los resultados en la segunda serie no es tan uniforme como la que se produce en el músculo esquelético (7). Podría argüirse que el tiempo necesario para que la fibra post ganglionar simpática degenere y no pueda ser excitada por las drogas estudiadas, fuese mayor que el de 72 a 110 horas empleado en la segunda serie. Se cree, no obstante, que éste no es el caso, ya que: a) No hay relación entre el tiempo de denervación del testigo y la inversión de los resultados, y b) Hampel (8) obtiene una hipersensibilidad de importancia en la m.n. ante la acción de la adrenalina a las

48 horas de extirpar el ganglio cervical superior. Los tiempos de 72 a 110 horas dejan, por tanto, un margen de seguridad bastante amplio. Esta diferencia de uniformidad podría explicarse a través de los factores siguientes:

a) Huidobro y Poblete (7) trabajaron con un músculo estriado y con un nervio motor; aquí se ha estudiado un músculo liso y un nervio de sistema autonómico.

CUADRO II

Efecto de las drogas sobre la membrana nictitante crónicamente denervada en comparación con la membrana testigo denervada horas antes del experimento.

Denervación		Extr. post-hipofisario		Prostigmina		Veratrina		Cl_2Ba	
horas	días	T.	C.	T.	C.	T.	C.	T.	C.
72	7		+		+		+		+
110	19	0		=		=		=	
72	19	=		+		+		+	
76	22	+		+		+		+	
96	24	+		+		+		+	
96	31	=			+		+		+
96	31		+		+	±	+	0	
100	34		+	=		=		=	

T.: Membrana nictitante denervada horas antes de la experiencia.
C.: Membrana nictitante denervada crónicamente (días) antes de la experiencia.
O. No se usó la droga.
+. Membrana nictitante que se contrae más intensamente.
=. Igual contracción en ambas membranas nictitantes.

b) La hipersensibilidad desarrollada por un músculo esquelético es mayor que la desarrollada por el músculo liso, como se ha podido comprobar, al menos para la acetilcolina; la hipersensibilidad de la fibra muscular estriada puede ser 1 000 a 10 000 veces mayor que lo normal. Esta diferencia entre los dos tipos de fibra muscular es explicado por algunos autores ([5] y [10]) suponiendo que el desarrollo de la hipersensibilidad estaría en relación directa con el grado de inactividad del efector que sigue a la denervación.

c) El grado de hipersensibilidad que presenta una estructura puede ser distinto para las diferentes drogas: Rosenblueth ([13]) señala que la sensiblidad por denervación de la m.n. es escasa para la fisostigmina, mayor para al acetilcolina y mayor aún para la adrenalina.

Teniendo presente lo ya discutido, la divergencia entre la primera y segunda serie experimental se explicaría de la siguiente manera: las drogas estudiadas excitarían tanto las fibras musculares lisas como las fibras nerviosas post ganglionares simpáticas. La suma de estas dos

acciones sería superior a la hipersensibilidad desarrollada por la denervación de la fibra muscular de la m.n. (ver resultados de la primera serie). Cuando se excluyen funcionalmente las fibras nerviosas post ganglionares, por un corto período de degeneración, podría exteriorizarse la hipersensibilidad desarrollada por la m.n. crónicamente denervada. Esta hipersensibilidad sería de muy escaso monto, por lo cual en la segunda serie disminuyen los casos en que la m.n. de menor tiempo de denervación es más sensible y aumentan los casos en que la m.n. crónicamente denervada iguala o supera la contracción de su congénere.

Los resultados obtenidos llevan a pensar, entonces, que el extracto post hipofisario, prostigmina, cloruro de bario y veratrina pudieran estimular las fibras nerviosas post ganglionares simpáticas. Muy recientemente Ambache ([3]) describe que el cloruro de bario es capaz de estimular el ganglio cervical superior, hecho que no hace imposible la suposición planteada. En todo caso tal posibilidad necesitaría una confirmación directa, que sería de suyo interesante. De ser así, sería también interesante revisar las excepciones descritas a la ley de denervación, ya que ellas podrían ser más bien aparentes que reales, como se estima que ha sucedido en las observaciones referidas por otros autores ([7]) y en las aquí referidas.

Del examen de los resultados puede deducirse, además, la posibilidad de que el nervio autonómico tuviera otras influencias funcionales sobre la actividad del efector, aún no bien precisadas. Así, el extracto post hipofisario produce relajaciones puras del músculo liso de la m.n. sólo en las membranas crónicamente denervadas y no en los testigos de la primera serie, que conservan su nervio sin degenerar. Este hecho debe ser subrayado ya que no se ha descrito anteriormente modificación cualitativa de una acción farmacológica por la denervación del efector.

Resumen y Conclusiones

En gatos anestesiados con pentobarbital sódico se estudia el efecto del extracto post hipofisario concentrado en ocitosina, prostigmina, cloruro de bario y veratrina sobre la membrana nictitante aguda y crónicamente denervada.

Se encuentra que la membrana nictitante agudamente denervada es más sensible que la crónicamente denervada (4 a 73 días) a la acción de las drogas señaladas.

Si se compara la sensibilidad de la membrana nictitante crónicamente denervada entre 7 y 34 días con su homóloga contralateral denervada entre 72 y 110 horas antes, se puede apreciar que la primera es más sensible a la acción de las mismas drogas.

Se discute esta acción y se concluye que los fármacos mencionados podrían estimular las fibras simpáticas post ganglionares.

Summary

The effect of pitressin, neostigmine, barium chloride and veratrine was studied on the acute and chronically denervated nictitating membrane of cats anesthetized with sodium pentobarbital.

The nictitating membrane acutely denervated (control) is more sensitive than the chronically denervated (4 - 73 days) to the action of these drugs, but the chronically denervated (7 - 34 days) is more sensitive than the contralateral denervated 72 - 110 hours before the experiment.

These effects are discussed. As a tentative explanation a possible excitatory action of these drugs on the post-ganglionic sympathetic fibres is suggested.

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Se exponen a continuación algunas abreviaturas comunes:

metro	m	litro	l	microgramo	µg
centímetro	cm	centímetro cúbico	cm^3	gamma	γ
milímetro	mm	mililitro	ml	por ciento	%
micrón	µ	kilogramo	kg	hora	h
milimicrón	mµ	gramo	g	minuto	m
Angström	A°	miligramo	mg	segundo	s
				milisegundo	ms

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